TROPHIC RELATIONSHIPS OF PREY SPECIES (*ETRUMEUS WHITEHEADI,* MYCTOPHIDAE AND *SEPIA)* AND THEIR PREDATORS (*MERLUCCIUS CAPENSIS, MERLUCCIUS PARADOXUS, LOPHIUS VOMERINUS* AND *TRACHURUS CAPENSIS)* OFF NAMIBIA

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Abstract

This study was conducted in Namibian waters that forms part of the Benguela current ecosystem. This upwelling-driven ecosystem supports high abundances of fish species, some of which have been the backbone of Namibian commercial fishery for decades. The study of the trophic relationships of prey species of commercial fish is important, as it improves understanding of the ecology of their predators and considers multi-species interactions in fisheries management. The objectives of this study were to assess the trophic levels of *Sepia* spp, *Etrumeus whiteheadi* and Myctophidae, to determine the trophic relationships among these species, to identify their potential trophic roles in the marine ecosystem and to determine the likely contributions of these prey species to the diet of *Merluccius capensis, Merluccius paradoxus, Lophius vomerinus* and *Trachurus capensis,* using stable isotopes. Muscle tissues were sampled from *E. whiteheadi*, Myctophidae, *Sepia* spp*, M. capensis, M. paradoxus, L. vomerinus* and *T. capensis*.

Isotope analyses revealed that all the prey species analysed are on the same trophic level except for *L. hectoris* that fed at a slightly higher trophic level. $\delta^{15}N$ values of prey species varied among all prey species. *Symbolophorus boops* had the most depleted $\delta^{15}N$, while *L. hectoris* had the most enriched $\delta^{15}N$ values. Significant differences were noted in δ13C', with *D. hudsoni* having the most depleted and *E. whiteheadi* the most enriched δ13C'. Isotope-based population metrics showed over lapping of trophic niches of all species, with *S.boops* having a significantly wider trophic niche.

All prey species analysed are important in the ecosystem since they all contributed to the diet of the four predators, although their contribution varied. A Bayesian isotope mixing

model showed no significant difference in relative contribution of prey an indication that prey availability is possibly a greater determining factor of prey dietary contribution. *Etrumeus whiteheadi* was a dominant prey item in the diets of the predators with an exception of that of *M. paradoxus*.

This study contributes towards understanding of prey trophic interactions, which can aid the implementation of an ecosystem approach to fisheries management in Namibia.

Key words: stable isotopes, trophic niche, trophic relationships, trophic level, prey species, carbon, nitrogen, *Etrumeus whiteheadi*, Myctophidae, *Sepia* spp.

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Abbreviations

ANOVA: analysis of variance

EMS: electro mass spectrometer

FV: Fishing vessel

GC: Gas combustor

NM: nautical miles

RV: Research Vessel

SEA_C: Standard ellipse area

SIA: Stable isotope analysis

Tukey HSD: Tukey Honest Significant Difference

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Dedication

I dedicate this work to my late father, Tate Augustus “Aukus” Erasmus Nuule. Father although you are physically not here, I know that you never really left because you are forever in my heart and for that reason, I have done everything knowing that you have been watching over me.

_____Continue to rest in eternal peace until we meet again_____

Declarations

I, Victoria Ndinelago Erasmus, declare hereby that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.

…….. Date……

(Victoria Ndinelago Erasmus)

Chapter 1

Introduction

1.1 Background

The Benguela current upwelling along the Namibian coast is known to be extremely productive (Heymans *et al.*, 2004; Cochrane *et al.*, 2009). It supports an important high biomass of zooplankton, as well as high abundances of pelagic and demersal fish species due to its high primary production (Shannon & O'Toole, 1998; Sumaila, Boyer, Skogen, & Steinshamn, 2004; Shannon, Hempel, Malanotte-Rizzoli, Moloney, & Woods, 2006; Cochrane *et al.*, 2009). It is one of the eastern boundary current systems of the World's oceans situated along the West Coast of Africa in the South-East Atlantic Ocean. The Benguela current ecosystem can be divided into two major ecosystems; the southern Benguela off the west coast of South Africa and northern Benguela off the coast of Namibia (Heymans, Shannon, & Jarre, 2004). The living marine resources of the Benguela ecosystem have been exploited for nearly a century (Hamukwaya, 1999; Heymans *et al.*, 2004).

The Benguela current ecosystem has a mean annual primary productivity of about 1.25 kg C/m^2/year (Heymans *et al*., 2004). The marine waters off Namibia support a large biomass of marine species, aiding complex trophic linkages due to their high level of upwelling based primary production (Hutchings *et al.* 2009). Heymans *et al.* (2004) documented several dramatic changes such as overfishing and physical challenges that

happened to the northern Benguela over the years. This includes high catches sustained by this system in the 1970s, as well as large populations of some planktivores, mainly *Engraulis encrasicolus* (anchovy) and *Sardinops sagax* (sardine). Furthermore, they noted that transfer efficiencies to higher trophic levels were highest during the 1980s. In addition there was evidence of severe stress on the northern Benguela system, since catches were lower and omnivory was reduced (Heymans *et al*., 2004). In addition there was changes in most of the energy that flowed through pathways, as it was not transferred as efficiently up the trophic chain as in the 1980s (Heymans *et al.*, 2004). Despite such major changes, the general energy flow pathway in the northern Benguela ecosystem remains as: primary production → zooplankton → pelagic fish → demersal fish, with pelagic and demersal fish acting as the secondary consumers (Heymans & Baird, 2000). Small fishes such as *Etrumeus whiteheadi*, Myctophids as well as *Sepia* spp. (cuttlefish) occupy different trophic positions within the food web, causing a network of trophic links (Iitembu, 2014; Staby & Krakstad, 2008). These small species are mostly preyed on by many commercially important species. It is important to study the different trophic relationships among these prey species, since important Namibian commercial species such as *Merluccius paradoxus* (Franca, 1960), *Merluccius capensis* (Castelnau, 1861), *Trachurus capensis* (Castelnau), 1861) and *Lophius vomerinus* (Regan, 1903) and other predators that inhabit the Benguela current ecosystem feed on them (Bianchi, Carpenter, Roux, Molloy, Payne, Pillar, & Crawford, 1999; Walmsley, Leslie, & Sauer, 2005; Iitembu, 2014).

Merluccius paradoxus and *M. capensis* have been the most valuable demersal resources in the region since 1965 (Gordoa, Macpherson, & Olivar, 1995; Payne *et al.,* 2001). Annual hake (*M. paradoxus* and *M. capensis)* landings in Namibia, South Africa and Angola averaged 300,000 tonnes per year in 2000-2010, with over 70% of this being *M. capensis* (Benguela Current Commission [BCC], 2012). *Merluccius paradoxus* and *M. capensis* are economically the most important fish stocks in both Namibia and South Africa, worth about 5% of the Gross Domestic Product (GDP) in Namibia (MFMR & NPC, 2013). These species do not only account for most of fisheries' catches, but they are also important secondary consumers in the Benguela Current ecosystem (Iitembu, 2014), and influence its food webs.

Monkfish fish, in particular *L. vomerinus* is also a commercially valuable species in both Namibia and South Africa (Maartens & Booth, 2001; Walmsley *et al*., 2005; Fariña *et al*., 2008). The value of monkfish products in Namibia is especially high, in terms of price per unit weight, which makes it an important contributor to the country's economy (Maartens & Booth, 2001; Payne *et al.,* 2001; Sumaila *et al.,* 2004). In 1999, Food and Agriculture Organization (FAO) recorded ~21 000 tonnes of total catches for *L. vomerinus*, with the highest catches obtained in Namibia and South Africa (Fariña *et al*., 2008). Statistics have indicated an increase in catches of *L. vomerinus* from less than 2000 tonnes to 12 000 tonnes since 1994 (Booth & Quinn, 2006).

Cape horse mackerel (*T. capensis*) is listed among the five major species of commercial importance (Hamukwaya, 1999; Sumaila *et al.,* 2004) that dominates landings in Namibia, although of relatively low value (Hutchings *et al.,* 2009). Currently, *T.*

capensis is one of the biggest fisheries in terms of landed volumes in Namibia fishing sector because it is the most abundant commercial pelagic species in the northern Benguela (D'Almeid, Krakstad & Kanandjembo, 2001; Payne *et al*., 2001; Hampton, 2003).

According to several researchers (Carrasco, Perissinotto & Nel, 2012; Iitembu, Miller, Ohmori, Kanime, & Wells, 2012; Mohanraj & Prabhu, 2012; Iitembu, 2014), information on the diet of fishes is significant in understanding the basic functioning of fish assemblages. This information is extensively used for ecological work in addition to modeling and is becoming an increasingly important constituent in ecologically based management (Mohanraj & Prabhu, 2012). Information of feeding ecology presents useful insight into an organism's environment (Layman, Arlington, Takimoto, Quattrochi, & Montana, 2007) and is vital to the study of the ecology of individual species (Moody, 2007). Mohanraj and Prabhu (2012) recognized food as the key factor in all ecosystems such as aquatic environments. They stressed that food partitioning outline functional groups within the community, which get together in guilds according to trophic similarity. Food consumption has among other factors, the potential to yield information about the niche a species occupies in its habitat (Iitembu, 2014). It is also helpful in interpreting some of the higher level tropic relationships in ecosystems (Mohanraj & Prabhu, 2012). Furthermore, food is a primary component of the ecology of an organism. Information about species evolution over a period of time as well as, individual behaviour, and the role individuals and species play in communities and food webs can be obtained from knowledge about the food of organisms (Moody, 2007). The

prey taken by a predator represents significant information that can be used to explain trophic aspects such as niche breadth as well as competition for resources and spatial overlap (Hammerschlag-Peyer, Yeager, Araújo & Layman, 2011).

Merluccius paradoxus, *M. capensis*, *T. capensis* and *L. vomerinus* are some of the commercially important species in Namibia that feed on the prey species that are the subjects of this study. It is well documented that *Etrumeus whiteheadi*, Myctophidae species such as *Symbolophorus boobs* (Richardson, 1845), *Lampanyctus australis* (Tåning, 1932), *Lampanyctodes hectoris* (Günther, 1876), *Diaphus meadi* (Nafpaktitis, 1978), *Diaphus hudsoni* (Zurbrigg & Scott, 1976) and cuttlefish such as *Sepia elegans* (Blainville, 1827) and *Sepia australis* (Quoy and Gaimard, 1832) are preyed upon by these commercial species and other fish that inhabit both the northern Benguela ecosystem (Walmsley *et al.*, 2005; Iitembu, 2014) and the southern Benguela ecosystems (Punt, Leslie, & du Plessis, 1992; Shannon, Moloney, Jarre & Fiel, 2003). *Trachurus capensis* also feeds on some of these prey species, however, there is little knowledge existing concerning its prey and feeding ecology (D'Almeid, Krakstad, & Kanandjembo, 2001).

For these commercial species to continue thriving we need to understand their feeding ecology. Most studies investigating trophic relationships have used the common traditional approach of stomach content analysis. However, this method has shortcomings that can not be ignored. For instance, Carrasco *et al*. (2012) observed that demersal fish often regurgitated their prey when brought to the surface, thereby giving an impression of an empty stomach. Additionally, by the time the stomach content is

analysed, the food is likely to be digested making prey items unrecognizable and therefore difficult to identify (Mqoqi, Lipin´ski, & Salvanes, 2007; Carrasco *et al*., 2012). Probably, the major drawback of stomach content is that results from direct examination of stomach content only provide snapshots of the most recent feeding and is in general also spatially and temporally limited (Van Der Lingen & Miller, 2011; Carrasco *et al*., 2012; Iitembu, 2014). Since some species have large masses of durable hard parts that lead to differential rates of digestion, stomach content analyses may be biased (Tollit *et al*., 1997). It is in light of the above that ecologists are compelled to develop and utilize other techniques that can be used instead of or in combination with the traditional methods. In order to understand food webs, energy pathways and trophic interactions, researchers have been using several methods such as stomach content analysis and the use of fatty acids as biomarkers. Warren (1989) Kioboe *et al.* (1990), Bamstedt *et al*. (2000) and Trites (2001) have also identified other approaches such as faecal analysis, fatty acid biomarkers analysis (Schukat *et al*., 2013), radio tracer and immunological approaches. Although these methods have helped to shed more light and resolve food web structure, numerous drawbacks have been noted, so much that even if they can still be used, they need to be coupled with other methods such as stable isotope analysis. These known shortcomings compelled researchers to develop and employ alternative, indirect chemical analysis techniques that may more accurately reflect the long term diet and trophic dynamics of organisms (Herman, Burrows, Wade, Durban, Matkin, LeDuc, Barrett-Lennard, & Krahn, 2005).

The technique of stable isotopes analysis, especially those of nitrogen and carbon determines the relative trophic levels and trophic relationships of consumers (Peterson & Fry, 1987). Furthermore, the stable isotope analysis approach is based on the principle that carbon and nitrogen isotope ratios in animals' tissues closely reflect that of their diet, with a predictable enrichment factor averaging approximately 3.4 ‰ per trophic level (Peterson & Fry, 1987) for nitrogen and 1‰ per trophic level for carbon (Vander Zanden *et al.*, 1998; Post, 2002a). Therefore, stable isotopes of nitrogen and carbon have been the most widely applied in studies that elucidate ecological processes (Choy *et al.*, 2009; Stowasser *et al.*, 2009; Iitembu *et al.*, 2012).

In this study, isotope signatures of both carbon and nitrogen of fish tissues from *E. whiteheadi*, Myctophidae species and *Sepia* spp. were measured in order to assess their trophic interactions, to determine their trophic levels, to assess their trophic roles in the marine ecosystem, as well as to determine their relative contributions to hake, monkfish and horse mackerel diet. These prey species were chosen because they are considered as prey of Namibian commercial species like *M. paradoxus*, *M. capensis, T. capensis* and *L.vomerinus*, based on documented stomach content analysis studies (Bianchi, Carpenter, Roux, Molloy, Boyer, & Boyer, 1999).

1.2 Orientation of the study

Many studies that have analysed feeding ecology of commercially important species especially in the northern Benguela ecosystem have used stomach content analysis (Macpherson & Roel, 1987; Payne, Rose, & Leslie, 1987; Roel & Macpherson, 1988;

Macpherson & Gordoa, 1994; Traut, 1996). These studies have formed important foundations for most trophic knowledge and can be considered to be moderately good. Most of the known studies that have used isotope analysis to elucidate trophic interactions of organisms were only limited to commercial species (such as Iitembu, 2014 and Mohanraj & Prabhu, 2012). The aim of this study was to use stable isotopes measurements of nitrogen and carbon to resolve trophic aspects of the following non-commercial prey species: *E. whiteheadi, S. boops, L. australis, L. hectoris, D. meadi, D. hudsoni*, *S. australis* and *S. elegans.* Additionally the relative contributions of these prey species to the diets of commercially important species such as *M. paradoxus*, *M. capensis, T. capensis* and *L. vomerinus* was examined.

1.3 Statement of problem

Sepia spp., *E. whiteheadi* and Myctophidae are not commercially exploited species, but are prey of many commercially important species such as *M. paradoxus*, *M. capensis, T. capensis* and *L. vomerinus*. For the commercial species to continue thriving; ecologists need to have a good understanding of their feeding ecology. An excellent perceptive of natural history of organisms and of species interactions, is a required parameter in guiding ecologists to the most thorough understanding of food webs (Layman *et al.,* 2007). Fisheries resources have been for a long time, an important food source, source of income and a form of employment. Therefore, it is necessary to find better ways to manage these resources in order to maintain these benefits not only for this generation but also for future generations. FAO has formulated some of the principles and

approaches for effective and responsible fisheries management. These approaches relate to an ecosystem approach to fisheries (EAF) that should be adopted by fisheries managers (FAO, 2003). One of the central goals of this EAF is to ensure that resources are used sustainably to ensure that they benefit future generations (FAO, 2003). Many researchers have acknowledged the need for fisheries managers to move towards an ecosystem approach to fisheries management (Roux & Shannon, 2004; Sanchirico, Smith, & Lipton, 2006; Fulford, 2013; Iitembu, 2014). The implication of fishing commercial species without fully understanding their feeding ecology may have a negative impact on their sustainability and the stability of the food web. Insufficient monitoring and indirect human impact on these prey species could also negatively influence commercial fish stocks, employment and income generation and may subsequently lead to disruption of the entire fishing industry.

Assessing the trophic relationships of prey species (*E. whiteheadi*, Myctophidae and *Sepia* spp.), their trophic levels and their role in the marine ecosystem is vital for the sustainability of the fishery. It would provide improved knowledge of prey species' relative position in the food web and help ecologists in determining their specific role in this marine ecosystem. These prey species (*E. whiteheadi*, Myctophidae and *Sepia* spp.) were selected because they are prey for Namibia's commercially important speces (based on stomach content analyses), additionally, they are used as bait for fishing and also used in fish meal production. Furthermore, these species are poorly studies.

1.4 Objectives of the study

Objectives of this study were to:

a) Assess the relative trophic levels of *Sepia* spp., *Etrumeus whiteheadi* and Myctophidae.

b) Determine the trophic relationships among *Sepia* spp., *Etrumeus whiteheadi* and Myctophidae.

c) Identify the potential trophic roles of *Sepia* spp., *Etrumeus whiteheadi* and Myctophidae in the marine ecosystem.

d) Determine the relative importance of *Etrumeus whiteheadi*, Myctophidae and *Sepia* spp. as prey of hake, monkfish and horse mackerel.

1.5 Hypotheses of the study

a) Round herring (*Etrumeus whiteheadi*), lanternfishes (Myctophidae) and cuttle-fishes (*Sepia* spp.) are on the same trophic level. They are secondary consumers in the marine ecosystem and possibly also feed on the tertiary consumer level.

b) Round herring (*Etrumeus whiteheadi*), lanternfishes (Myctophidae) and cuttle-fishes (*Sepia* spp.) are crucial in the food web, since they form a link between primary and tertiary consumers and play an important role in the energy flow within the system.

c) Round herring (*Etrumeus whiteheadi*), lanternfishes (Myctophidae) and cuttle-fishes (*Sepia s*pp.) contribute equally to the diets of hake, monkfish and horse mackerel.

1.6 Significance of the study

The Namibian Ministry of Fisheries and Marine Resources has been trying for decades to implement a knowledge-based ecosystem approach to fisheries management. Adopting such an approach requires an accurate understanding of the trophic ecology among the fish communities in the food web. There is a common understanding among fishery managers and scientists that new alternative approaches are required to better manage fisheries resources at ecologically sustainable harvest levels (Roux & Shannon, 2004; Sanchirico, Smith & Lipton, 2006; Fulford, 2013; Iitembu, 2014). This need exists because fisheries ecologists are increasingly becoming more aware of the importance of managing whole ecosystems rather than single resources (Officer & Parry, 1997). Hobson & Welch (1992), also emphasized that management of a fishery requires a thorough understanding and detailed knowledge of the trophic relationships between the species involved as predation does not only have a significant influence on community structures but it also greatly influence population dynamics. Quantification of trophic linkages among various species was identified as vital for an ecosystem approach to fisheries management since these data support the development of different food-web models that allow fishing pressure to be analysed in an ecological framework with other sources of mortality (Christensen & Pauly, 2004). Mohanraj and Prabhu (2012) emphasized that knowledge of the feeding ecology of non-commercial as well as commercial species is necessary for implementing a multispecies approach to fisheries management.

This study is one of the few trophic studies on prey species through the use of stable isotope analysis in the Benguela ecosystem (Van Der Lingen & Miller, 2011; Huenerlage & Buchholz, 2013; Schukat, Auel, Teuber, Lahajnar, & Hagen, 2013) and the only known study in the northern Beguela ecosystem. The findings of this study will largely contribute toward understanding of trophic relations in Namibian waters. Consequently, this can aid the implementation of an ecosystem approach to fisheries management in the Northern Benguela Current region and it could be extended to other marine ecosystems. Although the species under study are not of any commercial importance, they are preyed upon by commercial species. The prey species of our commercially important species are still relatively poorly studied and there exist knowledge gaps in understading their relative contribution to the diet of predators as well as their trophic levels. Apart from insufficient knowledge of prey species, major changes have occurred in the northern Benguela system, including spatial changes in the distribution of small pelagic fish, which are preyed upon by commercial species (Shannon *et al.*, 2003; Shannon & Cury, 2004), and increases in biomass of other species (Utne-palm *et al.*, 2010; Flynn *et al*,. 2012; Roux *et al.*, 2013) which could be competing for food sources with commercial species. This necessitates continuous studies to monitor the impacts of these changes on the trophic dynamics of the northern Benguela system and to ensure creation of a long term data series (Iitembu, 2014). It is well understood that the longer the time series, the more accurate the data and interpretation thereof. The results obtained in this study will therefore complement the

efforts of the Ministry of fisheries and marine resource to successfully implement the Ecosystem approach to fisheries management of its commercial resources.

Chapter 2

Literature review

2.1 The Benguela current marine ecosystem

The Namibian marine waters within 1500 km of the Namibian coastline are characterized by the cold, nutrient rich, Benguela current with seasonal upwelling (Bianchi *et al.,* 1999; Bloyer *et al.,* 2000). The Benguela current ecosystem has been constantly subjected to both climate and environmental change over the past years (Shannon & Nelson, 1996). The Benguela Current is loosely considered to envelop the south-west Atlantic coast of Africa from the continental shelf between the Angola-Benguela frontal zone off northern Namibia and the Agulhas retroflection area, typically between 36 and 37°S (Lutjeharms & Meeuwis, 1987; Shannon & O'Toole, 1998; Cochrane *et al.,* 2009) (Fig. 1). As such, it covers the west coast of South Africa, the entire Namibian coast, and part of southern Angola. The Benguela Current meets the warmer Aghulhas Current flowing east from the Indian Ocean and in the north it interacts with the tropical warmer Angolan current flowing south (Bianchi *et al.,* 1999). Thus, the Benguela Current is bounded by two warm-water regimes, making it unique amongst the world's upwelling systems (Shannon 1985; Hutchings *et al.,* 2009; Veitch, Penven, & Shillington, 2009).

The Benguela Current is characterized by an intense equator-ward flow and high levels of south easterly wind-driven coastal upwelling (Nelson & Hutchings, 1983; Carr & Kearns, 2003). The surface currents of the Benguela ecosystem are therefore generally

equatorward, with vigorous coastal upwelling cells, strong and narrow equator-ward shelf edge jets (Bianchi *et al.*, 1999; Shannon, *et al.*, 2006). Coastal upwelling is a process whereby deep water which is rich in nutrient is brought to the surface by currents.

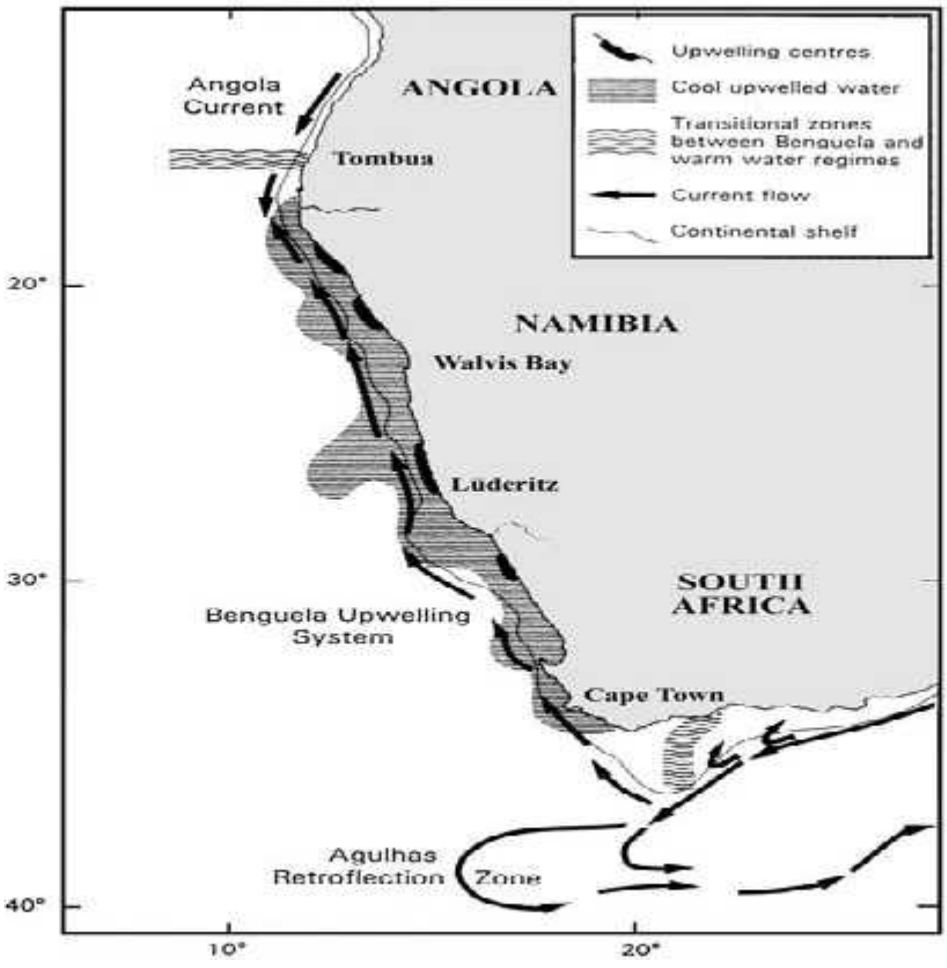

Figure 1: Map of the northern Benguela area off the Namibian coast (adopted from FAO, 1999)

Off Namibia, upwelling is particularly strong during the cooler months, which reinforces the seasonal effect and causes a definite temperature cycle (Bianchi *et al.,* 1999; Gordoa *et al*., 2000). The well documented highly productive upwelling of the Benguela ecosystem that presents nutrient-rich waters and ideal thriving conditions for populations of fish, seabirds and mammals is a direct result of the mingling of warm and cold currents of the Benguela ecosystem (Cochrane *et al*,. 2009). Essential nutrients are transported from the deeper waters to the ocean surface as a direct result of this upwelling (Garrison, 1999).

2.2 Trophic relationships

Organisms within an ecosystem feed on one another, forming food chains and food webs. A food chain represents a succession of organisms that eat another organism and are, in turn, eaten themselves. A trophic level of a species points out the average number of times chemical energy is transformed from a consumer's diet into a consumer's biomass along the food chains that culminates into the species (Williams & Martinez, 2002) and their efficiencies of transferring material and energy (Shannon *et al.*, 2003). As such, trophic levels can be represented by numbers, for instance starting at level 1 with plankton as primary producers that form the base of a food chain as they directly "gather" energy from the sun and fuel such a food chain. Advance trophic levels are assigned numbers subsequently based on how distant the organism is along the food chain (Fig. 2). This implies that food chains are pathways of organic energy through trophic positions from the base to the top of a food web (Post, 2002a). Armstrong (2001)

stated that trophic studies yield masses of information such as the amount of energy lost or transfered at each link (trophic level) in the food chain. Armstrong (2001), emphasized that the more links a food chain has, the more dissipated or unusable energy can be found in the chain, because energy initially stored by primary producers at the base of the food chain is dissipated as one moves along the food chain.

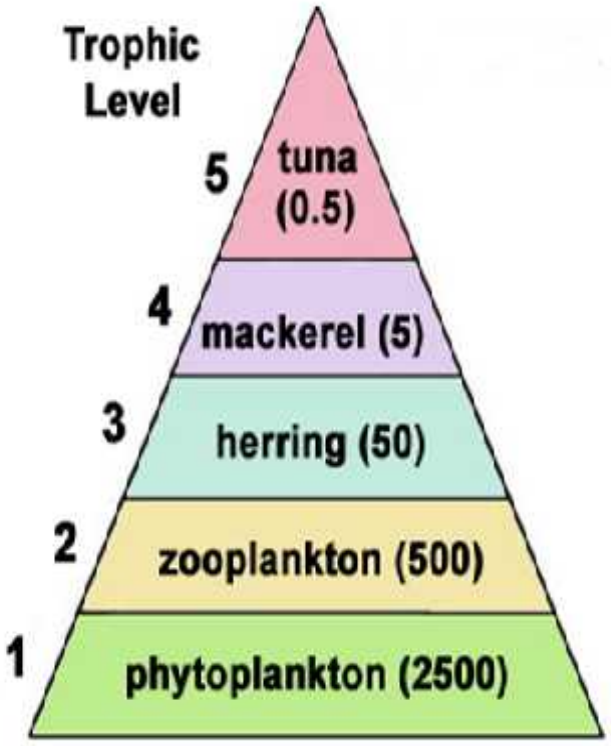

Figure 2: A generalized aquatic food pyramid indicating trophic levels (source: Armstrong, 2001).

There is generally a 90% loss at each link of the food chain, creating a pyramid-shaped energy diagram (Fig. 2) that is wider at the bottom and narrow at the top (Armstrong, 2001). Other researchers, through the study of food webs were able to shed light on carbon sources of different food webs, contributions of possible prey species to the diet of their consumers as well as the dietary preference of these consumers (Herman *et al*., 2005; Carrasco *et al*., 2012; Iitembu, 2014).

Food webs were used theoretically by the earliest ecologists (Darwin, 1859; Hardy, 1924; Elton, 1927) and are still requested in present-day ecological theoretical studies (Officer & Parry, 1997). Although food webs are composed of a network of organisms that are in turn linked together by their trophic interactions, the strength and significance of these interactions vary (Paine & Schindler, 2002). Organisms in an ecosystem often trophically interact in complex ways mostly because some organisms feed at more than one trophic level, while sometimes trophic levels of some organisms change as they grow (Werner & Gilliam, 1984; Mohanraj & Prabhu, 2012). Such elements affect the task of resolving trophic interaction among organisms, making it a difficult one (Hobson & Welch, 1992). Trophic studies of marine communities is especially daunting, for instance trophic studies of upper-trophic level consumers, because year-round sampling of different components of the food web is highly problematic (Cortés, 1999). Similarly, a large quantity of information is often required to assess the trophic interactions in a food web and the development of trophic models is further complicated by the fact that these interactions change over time and space (Shannon *et al.*, 2003; Iitembu, 2014). Despite such challenges, understanding of the trophic relationships among marine

species is indoubtly fundamental to managing our marine resources with confidence (Yodzis, 1994; Mueter & Megrey, 2006). This can be achieved through a science-based ecosystem approach to fisheries management that places multi-species trophic interactions into great consideration (Cochrane *et al.*, 2009; Paterson & Petersen, 2010; Shannon, Jarre, & Petersen, 2010).

Apart from the use of stomach content analysis as the traditional approach, trophic interaction models (Berryman, 1992; Giacomini *et al.*, 2013) such as the Lotka-Volterra model (Lotka, 1925), ecosystem dynamic models such as Ecopath (Pauly *et al.*, 2000) and High trophic level models such as larval individual-based models (Hermann, Hinckley, Megrey, & Napp, 2001) have greatly improved our understanding of trophic interactions in marine ecosystems. Stable isotope data are complementary to the development of these network models of food webs because they are time integrated and they provide data on the relative magnitude of entire trophic pathways down to sources of primary production (Peterson & Fry, 1987).

Over the last two decades, the use of stable isotope analysis, as a quantitave technique, and to some extent, the analysis of fatty acid signatures in ecological research has rapidly increased and diversified (Schmidt *et al.*, 2006; Post *et al.*, 2007; Koussoroplis *et al*., 2011). By measuring the isotopic concentrations of tissues of a suite of consumers, it is possible to determine relative trophic positions within a marine community (Hobson & Welch, 1992; Iitembu, 2014). The stable isotope analysis method provides temporally-integrated measures of trophic relationships among species and can detect trophic interactions not observed through stomach content analyses (Iitembu, 2014;

Herman *et al.,* 2005) and other earliest techniques such as feacal analysis, since they present only a snapshot of the most recent meal and may therefore not be representative of the typical long-term diet.

2.3 Prey species under study

Commercially important species such as *M. paradoxus*, *M. capensis*, *T. capensis* and *L. vomerinus* together with their prey depend on the highly productive Benguela upwelling ecosystem. These commercially important species prey on various small fishes such as *Sepia* spp., *E. whiteheadi* and Myctophidae species (Bianchi *et al.*, 1999; Iitembu, 2014). The trophic interactions of prey species and their predators through food webs play a major role in the structuring of aquatic systems (Vander Zanden, Shuter, Lester, & Rasmussen, 2000; Heithaus *et al.,* 2008). Determining the trophic level where a particular species feeds as well as identifying how much that particular species contributes to the diet of its predators are some of the critical aspects of assessing trophic interactions (Bowles, Schulte, Tollit, Deagle & Trites, 2011). By studying prey species ecologists might obtain detailed understanding of the ecology of their predators. Such knowledge is essential in formulation of models which describe energy pathways in the ecosystem.

2.3.1 *Etrumeus whiteheadi*

Etrumeus whiteheadi (Wongratana, 1983) (Fig. 3) is commonly known as round herring and belongs in the *Clupeidae* family. The Clupeidae family includes red eye herrings, shads, sardines, hilsa, and menhadens which includes many of the most important edible fishes in the world. *Etrumeus whiteheadi* is a marine, pelagic species that occurs mainly inshore and is found to a depth of about 200 m (Bianchi *et al.,* 1999; Sumaila *et al.,* 2004). This species has been found, as a bycatch, in both pelagic and demersal trawls (Sumaila *et al.,* 2004). It is found in the southeast Atlantic from Walvis Bay on the west coast off Namibia to Durban in South Africa, (Waldron, Proosch, & Armstrong, 1991; Heemstra & Heemstra, 2004; Bianchi *et al.,* 1999). Bianchi *et al.* (1999) stated that *E. whiteheadi* grows up to 20 cm. *Etrumeus whiteheadi* is fished and targeted by line anglers and used or sold as bait for fishing (Gil, 2005; Lindsay, 2006). It is also used for the production of fish meal and fish oil and is an essential prey for commercial species. van der Lingen (2003) foresees a possibility of *E. whiteheadi* to be canned if it is caught by vessels that have cooling facilities. The main food item for *E. whiteheadi* is reported to be zooplankton (James, 1988; Sumaila *et al.,* 2004). According to a stomach content analysis study of monkfish in the southern Benguela ecosystem, *E. whiteheadi* was listed to form part of the diet of both juveniles and adult monkfish accounting for 2.4% and 7.3% of the diet respectively (Walmsley *et al.,* 2005). They were also in the diet of both *M. paradoxus* and *M. capensis* (Pillar & Barange, 1997). Currently the species is only caught in small quantities, although the fishery is being expanded and experimental licenses for a redeye-directed fishery have been granted during 2010 in the Southern

Benguela, (Shannon & Shin, 2011). Because *E. whiteheadi* is currently not of commercial importance in Namibia, there is limited information on the species from this region (Staby & Krakstad, 2008).

Figure 3: *Etrumeus whiteheadi*

2.3.2 Myctophidae

Myctophidae (abbreviated as myctophids) is a family of fish commonly referred to as lanternfishes. In Namibia, myctophids includes *Diaphus hudsoni* (Zurbrigg & Scott, 1976), *Diaphus meadi* (Nafpaktitis, 1978), *Lampanyctus australis* (Tåning, 1932),

Lampanyctodes hectoris (Günther, 1876) and *Symbolophorus boobs* (Richardson, 1845). Myctophids (Fig. 4) are found at mid-water depths of 250 – 500 m (Bianchi *et al.,* 1999). The maximum size of most of these species is less than or around 10 cm, but a couple of species reach 20 to 25 cm standard length (Bianchi *et al.,* 1999). It is well known that myctophids are often one of the key elements of the oceanic food chains and food webs, mainly because they are significant predators of zooplankton (Staby & Krakstad, 2008; Gibbons, 1999; Connan, Mayzaud, Duhamel, Bonnevie, & Cherel, 2010). They are also major species in the aquatic ecosyetem because they are important food to commercially important and protected marine vertebrates (Wang & Chen, 2001). Salmon, tuna and fur seals are some of the species that prey on myctophids (Wang & Chen, 2001). Some of the commercially important species in the Beguela ecosystem that prey on myctophids are *T. capensis* (Konchina, 1986), *M. paradoxus*, *M. capensis,* and *Genypterus capensis* (Bianchi *et al.,* 1999; Iitembu, 2014).

Figure: 4 Myctophids

2.3.2.1 *Lampanyctodes hectoris*

Lampanyctodes hectoris is a small pelagic fish that grows up to a size of 6 cm (Bianchi *et al.*, 1999). The most frequently described lanternfish is *L. hectoris,* also the most prominent pseudo oceanic myctophid species described for the Benguela region (Hulley, 1986; Hulley, 1992). *Lampanyctodes hectoris* is a potential commercially important species that can be used for the production of fish meal (Bianchi *et al.,* 1999). In South Africa, *L. hectoris* is a target species caught in purse seine fishery, while in Namibia; this species is only recorded as a bycatch (Staby & Krakstad, 2008). In the northern Benguela, *L. hectoris* has been reported on the outer shelf edge more than 30 miles offshore and also near the Lüderitz coast, south of Walvis Bay and around the Cape Frio

area (O'Toole, 1976; Cruickshank 1982; Staby & Krakstad, 2008). *Lampanyctodes hectoris* was among the prey items identified in the stomach of both *M. paradoxus* and *M. capensis* (Pillar & Barange, 1997).

2.3.2.2 *Diaphus hudsoni*

This species is common in mid-water trawls, found at depths below 250 m, and grows up to 8 cm standard length (O'Toole, 1976; Bianchi *et al.,* 1999). *Diaphus meadi* is common prey species for *M. paradoxus*, *M. capensis*, *T. capensis* and *G. capensis* (Hulley, 1990; Bianchi *et al.,* 1999; Kainge, 2002). This species is one of the abundant myctophids in the Benguela current ecosystem (Olivar, 1987).

2.3.2.3 *Diaphus meadi*

Diaphus meadi were found in mid-water trawls and purse seines. It is found over the continental shelf and slope above depths of 250 m at night. It is commonly taken as prey by *M. paradoxus*, *M. capensis, T. capensis* and *G. capensis* (Bianchi *et al.,* 1999).

2.3.2.4 *Lampanyctus australis*

Lampanyctus australis are generally found far offshore over the outer edge of the continental shelf and upper regions of the slope generally deeper than 500 m. *Merluccius paradoxus*, *M. capensis, T. capensis* and *G. capensis* were listed as some of the important predators of this species (Bianchi *et al.*, 1999).

2.3.2.5 *Symbolophorus boobs*

Symbolophorus boobs is a bathypelagic species, found offshore between 18^0S and 25^0S in the northern Benguela (O'Toole 1976; Hulley, 1990; Bianchi, *et al*. 1999). *Symbolophorus boobs* is found in deep waters of 400 to 500 m far offshore (O'Toole 1976; Hulley, 1990; Bianchi, *et al*. 1999; Staby & Krakstad, 2008). Little is known about the ecology of this species.

2.3.3 *Sepia* spp.

All *Sepia* spp., commonly known as Cuttlefish belong to *Sepiidae*. Bianchi, *et al.*, (1999), reported seven species in Namibia and listed *elegans* (Blainville, 1827) and *australis* (Quoy and Gaimard, 1832) (Fig. 5) as two of the species. These species are reported to generally inhabit depths between 125 and 335 m (Bianchi, *et al.*, 1999; Jereb and Roper, 2005). They are found in the South Atlantic and occur mostly off Africa, but there are four species, which live in European waters. Of more than 20 sepiids in the southern African ecosystem, *S. australis* is reported to be the most abundant (Roeleveld, 1972). *Sepia* spp. are sometimes used as bait to catch demersal and semi pelagic fish (Gil, 2005; SPC 2009). They consumed by human and represent an important prey item for fur seals, skates and commercially important species such as *M. paradoxus* and *M. capensis* (Payne *et al*., 1987; Payne *et al*., 2001; Jereb & Roper, 2005). Based on the study of monkfish stomach content analysis, *Sepia* spp. were listed as forming part of the diet of both juveniles and adult monkfish fish accounting for 7.3% and 0.5%, respectively (Walmsley *et al.*, 2005). *Sepia* spp. was also found in stomachs of *M.*

capensis and *M. paradoxus* with 0.58% and 0.08 as percentage of occurrences, respectively (Pillar & Barange, 1997).

Figure 5: *Sepia australis*, one of the *Sepia* spp.

2.3.3.1 *Sepia elegans*

Sepia elegans is consumed by humans. *Sepia elegans* feeds mainly on molluscs, small crustaceans, fishes and polychaetes (Jereb & Roper, 2005; Mqoqi, Lipin´ski, & Salvanes, 2007). *Sepia elegans* is taken mainly as a by-catch in northern Benguela

(author observation) as well as in Mediterranean and West African trawl fisheries (Jereb & Roper, 2005).

2.3.3.2 *Sepia australis*

Based on the abundance of *S. australis* (Fig. 5) in both scientific surveys and commercial catches there has been indications that this species is sufficiently abundant to be exploited by fisheries and is seen to have potential commercial importance (Jereb & Roper, 2005). Euphausiid, megalopae, cephalopod and mysids were some of the main prey items identified in stomachs of *S. australis* in the southern Benguela (Gibbons, 1999; Mqoqi, Lipin´ski, & Salvanes, 2007). Mqoqi *et al.* (2007) also reported low intensities of cannibalism among *S. australis* species, which is caused by insufficient prey species in some regions. *Sepia australis*' diet compositionis influenced by prey availability rather then by prey preference, making this species an opportunistic feeder (Mqoqi *et al.,* 2007). Besides these limited studies, there is little knowl edge about the feeding ecology of *S. australis.*

2.4 Predators under study.

2.4.1 *Merluccius paradoxus* and *Merluccius capensis*

In the northern Benguela there are two main hake species: *M. capensis* (Fig. 6) and *M. Paradoxus* (Fig. 7). Hake is a bottom living species which live between 150 to 450 m depth (Bianchi *et al.,* 1999). They are opportunistic as well as predominantly piscivorous feeders (Sumaila *et al.,* 2004). Hake is a significant predator in the Beguela region and

thus influence on food webs (Macpherson & Roel, 1987). Stomach content analysis studies (Payne *et al.,* 1987; Traut, 1996; Pillar & Barange, 1997) in attempts to study the feeding ecology of hake concluded that hake is an important opportunistic feeder preying on available prey items. At zooplankton dominated habitats, hake is reported to feed mainly on large crustaceans (Pillar & Barange, 1993). In general, hake feeds on various demersal as well as semi-pelagic preys (Macpherson & Roel, 1987; Traut, 1996; Sumaila *et al.,* 2004) such as krill, crustaceans, cephalopods, myctophids (Iitembu, 2014; Bianchi *et al.,* 1999; Kainge, 2002), horse mackerel (Iitembu, 2014; Barange, Pillar, Huse & Hutchings, 2005), anchovy (*Engraulis encrasicolus*) (Barange *et al.,* 2005) and bearded goby, (Macpherson & Roel, 1987; Traut, 1996). Some studies also indicated intense cannibalism in hake species (Roel & Macpherson; kainge, 2002; Shannon *et al.,* 2003; Iitembu, 2014).

Figure 6: *Merluccius capensis*

Figure 7: *Merluccius paradoxus*

2.4.2 *Trachurus capensis*

Trachurus capensis (Fig. 8) is a mesopelagic species that feeds on several prey items such as pelagic gobies (Venter, 1976; Staby & Krakstad, 2008) and myctophids (Andronov, 1983). However, the diet of *T. capensis* is reported to change significantly as it grows (ontogenetic shifts) but generally, it is dominated by large calanoid copepods and euphausiids (Hutchings *et al.* 2009; Geist, Kunzmann, Verheye, Eggert, Schukat, & Ekau, 2014). Stomach content studies indicated that *T. capensis* feeds mainly on copepods and euphausiids (Barange *et al.*, 2005). Stable isotope analysis of nitrogen and carbon in *T. capensis* of the Benguela region have indicated that *T. capensis* is a secondary consumer and that its diet is dominated by euphausiids (Tjizoo, Ekau & Saint-Paul, unpublished).

Figure 8: *Trachurus capensis* (photo: Uanivi, 2014)

2.4.3 *Lophius vomerinus*

Lophius vomerinus (Fig. 9) is a bottom living species that inhabits waters of 200 to 400 m depth (Bianchi *et al.*, 1999; Sumaila *et al.*, 2004). It is an angler species that is opportunistic and non-selective in its feeding habits (Fariña *et al.*, 2008). *Lophius vomerinus* feeds on small fish such as red eye round herring (*E. whiteheadi*) and other fishes such as hake, (Dooley, Matsuura, Collette, Nelson, Fritzsche, & Carpenter, 2010; Sumaila *et al.*, 2004), *T. capensis* and pilchard on occasions (Bianchi *et al.*, 1999). Apart from the above mentioned prey items, monkfish can ambush any bottom living prey (Maartens, 1999). The information on the biology and ecology of *L. vomerinus* is still minimal (Maartens, 1999).

Figure 9: *Lophius vomerinus* (photo: Gamatham, 2011)

2.5 Methods of assessing trophic relationships

2.5.1 Stomach content analysis

Stomach content analysis is one of the traditional standard approaches for quantification of trophic relationships of organisms (Iitembu, 2014; Carrasco *et al*., 2012). The essential knowledge that laid the foundation for understanding ecological processes used today was primarily obtained from this conventional method. Pinnegar and Polunin (1999) identified presentation of ‘snap shots’ of dietary items in the stomach as one of the benefit of stomach content analysis. However, this is also a major downfall (Baker, Buckland, & Sheaves, 2013), in understanding diet preference and variation, especially

for species that are highly opportunistic in their foraging behaviour (Sholto-Douglas, Field, James, & van der Merwe, 1991).

Different prey items have different digestive rates, which is a source of bias in stomach content analysis (Parkins, 1993). Michener and Lajtha (2007) argued that some organisms digest their prey rapidly thereby deforming the morphology of the ingested prey, making identification difficult. Some ingested prey with fragile bodies such as zooplankton (Schukat *et al*., 2013) are assimilated quickly and are, therefore, infrequently found in the stomach thereby giving a false impression that such prey items where never present (Duffy & Jackson, 1986; Gee, 1989). In contrast, some prey items with hard bodies or parts such as fish otoliths are mostly, always found in stomachs (Duffy & Jackson, 1986; Gee, 1989), which may lead to over estimation of the significance of upper trophic-level prey such as fish (Hobson, 1993). In this case, stomach content analysis requires a knowledgeable taxonomist to identify nearly all the unidentifiable and partially digested organisms found in the stomach (Baker, Buckland & Sheaves, 2013). Some partially digested items can be difficult to identify, quantify and to separate into prey categories (Baker, Buckland & Sheaves, 2013). In some instances, some material is only ingested but does not guarantee that it is assimilated into the biomass of the organism (Michener & Schell 1994). Moreover, stomach structures differ considerably among species, presenting a problem in the definition of “stomach” contents, since it often includes material that is not assimilated e.g., bones, scales, and teeth. In this regard stable isotope analysis has an advantage over stomach content

analysis, since it distinguishes between assimilated food rather than ingested food (Michener & Lajtha, 2007; Phillips, 2012).

2.5.2 Behavioural studies in the laboratory

Earlier studies on predator-prey relationships often involved behavioural observations in the laboratory during analysis of feeding preferences of predators in order to understand animals' foraging behaviours. However, obtaining an adequate sample of prey items has been one of the limiting factors for such studies and laboratory studies produce at best a highly defective prediction of species relationships in the field (Ockelmann & Vahl, 1970; Feller *et al*., 1979; Boyd *et al*., 1984).

2.5.3 Compound specific isotopes

Michener & Lajtha, (2007) pointed out a derivative of GC–combustion interfaced–isotope ratio mass spectrometry (GC-C-IRMS) technology useful in ecological studies. This is a compound-specific isotope method referred to as ^{13}C -phospholipid fatty acid analysis (^{13}C -PLFA). And is one of the common techniques used in ecological studies (Michener & Lajtha, 2007). ^{13}C -PLFA involves quantification of ^{13}C-PLFA concentration (Michener & Lajtha, 2007). It involves using organic solvents to extract lipids, isolation of phospholipid by silicic acid chromatography, esterification of fatty acids by methanolysis, and separation of methylesters by capillary gas chromatography (GC) using a flame ionization detector (Tunlid, Ringelgerg, Phelps, Low & White, 1989). Since the late 1990s there have be a number of investigations into substrate usage

by different microbial communities using ^{13}C incorporation into fatty acids (e.g. Abraham, Hesse & Pelz, 1998; Hanson *et al*., 1999; Butler *et al*., 2004). This method was successfully used by researches such as Abraham *et al.* (1998), Butler *et al.* (2004) and Londry et al. (2004). Notwithstanding the great potential of fatty acids (^{13}C-PLFA method), especially in microorganisms, there are noted limitations. Michener & Lajtha (2007) listed two of the most common drawbacks for this method: (a) the identification database is currently limited to ~100 fatty acids; (b) details on explanation is complicated due to some markers that have more than one meaning. Additionaly, there are always fatty acid contaminants can lead to inaccuracy of results (Tunlid *et al.*, 1989).

2.5.4 Fatty acid signature

Ecologists have used fatty acid signatures in trophic studies to assess trophic relationships of *M. capensis* and *M. paradoxus* (Iitembu, 2014). In another study, fatty acids were used in combination with stable isotope analysis to analyse trophic interactions of calanoid copepods in the Benguela upwelling system (Schukat *et al*., 2013). Fatty acids are used as metabolic energy reserves and are accumulated in substantial amounts by many aquatic organisms (Pond, 2012). Fatty acids from ingested food are known to accumulate in the predators' tissue and some specific fatty acid classes can be used as indicative biomarkers for species of different trophic levels (Kirsch *et al.*, 2000). Fatty acids are transferred, without major modifications, through food chains, all the way from phytoplankton to consumers thereby preserving the dietary signature (Iitembu, 2014; Schukat *et al*., 2013). Most consumers lack the denaturize

enzymes required to biosynthesize unsaturated fatty acids and consequently have to acquire these components from their food, this makes fatty acids ideal diet tracers (Tocher & Ghioni, 1999). Fatty acid signature provide more detailed information on trophic aspects of organisms and their values represent true biochemical values because such values are not derived from equations. In a study by Iitembu (2014) significant information on hake's trophic relationships with demersal sharks could be obtained by the use of combined fatty acid analysis and stable isotope analysis. Currently there are three noted limitations of fatty acid analysis; firstly the identification database is currently limited to 100 fatty acids and secondly, there is a lack of understanding of how individual fatty acids change through elongation and desaturation processes occurring throughout a food web and finally, information of the use of fatty acids to investigate trophic relationships at higher trophic levels is stil unclear (Iitembu, 2014).

2.5.5 Immunological methods

Immunological methods have also been used to characterize aquatic food webs (Boreham & Ohiagu, 1978; Feller *et al*., 1979, 1985). These methods involve developing antisera from whole organism extracts, followed by double immune-diffusion precipitin tests of antiserum specificity. Antisera are usually taxon-specific and thus can be used to trace trophic relationships. This method is suitable for investigating organisms whose stomach contents cannot be visually identified. Feller *et al*., (1985) used this technique to investigate deep sea food web structure, where changes in water pressure frequently deform organisms and make gut contents difficult to identify. However, this method is

restricted to the specificities and number of antisera developed and is strictly qualitative. For systems with a large number of species, it would be expensive, time-consuming and impractical to explore all possible antisera (Michener & Lajtha, 2007).

2.5.6 DNA identification techniques

Michener & Lajtha (2007) identified a molecular approach that involves the use of deoxyribonucleic acid (DNA) identification techniques. The studies on quantification of proportion of prey consumed can be determined through analysis of faecal DNA using real-time PCR (Polymerase Chain Reaction) (Bowles *et al.* 2011). These are revolutionising stomach contents studies. An advantage of this technique is that species relations are identified and preserved; isotopes track element flows much less directly than species interactions (Michener & Lajtha, 2007). A drawback of DNA identification techniques is that they are mostly non quantitative, and this presents limits in their usefulness for some applications.

There are other conventional methods as well, such as radio telemetry or scat analysis used to study animals and ecological impacts of invasive species (Bodey, Bearhop & McDonald, 2011). These techniques can however be time consuming, laborious and costly.

2.5.7 Stable isotopes

2.5.7.1 What is a stable isotope?

Isotopes are atoms of the same element, that have the same number of protons and electrons, but differing numbers of neutrons in their nucleus, however, they have the same atomic number and the same chemical reactions (Michener & Lajtha, 2007). Isotopes therefore exhibit variations in atomic weight based on differences in the number of neutrons in the nucleus (Jardine, McGeachy, Paton, Savoie & Cunjak, 2003). Chemists designated atomic formulas to isotopes, for instance, naturally, nitrogen has two stable isotopes, ^{14}N which is the most common one and ^{15}N, the least common (Hoefs, 1997). Michener and Lajtha (2007), defined stable isotopes as those that are energetically stable, do not emit radiation and do not decay; thus, they are not radioactive which make them useful natural tracers. An isotope is said to be stable when the number of neutrons (N) and the number of protons (Z) are similar. There are about 300 known stable isotopes and more than 1200 radioactive isotopes (Hoefs, 1997). Although stable isotopes occur naturally in the environment, it is often at low levels (Rundel & Ehleringer, 1989) and they are distributed according to specific biological, geological, and chemical processes.

2.5.8 Use of stable isotopes in feeding ecology studies

There are several reasons for preference of the use of stable isotope analysis (SIA) methods over the the traditional methods. SIA is a valuable tool in a wide range of disciplines such as plant and animal ecology as well as environmental fields (Lepoint,

Dauby & Gobert, 2004), ecosystem gas exchange (Yakir & Sternberg, 2000), paleocology, watershed hydrology, forensics, soil nitrogen isotope composition studies and in the study of biology of modern and fossil vertebrates (Michener & Lajtha, 2007). All of the above mentioned applications take advantage of natural variations in stable isotope ratios, for instance, ^{13}C, which is the ratio of ^{13}C to ^{12}C relative to a reference standard, that result from the chemical or biological processes that cause isotopic discrimination (fractionation). Isotopic discrimination is a change in the ratio of heavy to light isotopes in a compound that results after uptake, processing or transformation (Post *et al.,* 2007).

The isotopes of lighter elements dominate isotope use in ecological research not only because biological compounds are dominated by these lighter elements, but also because the percent increase in mass caused by the addition of a single neutron is greatest for these elements (Michener & Lajtha, 2007). Thus far, ecologists have identified carbon, nitrogen, hydrogen and sulphur (Table 1) as the four main elements used in stable isotope analysis (SIA) for ecological research (Jardine *et al.,* 2003; West *et al.,* 2006). Ratios of stable isotopes of carbon, nitrogen, hydrogen and sulphur differ among various substances such as animal tissues. These differences allow for dietary assumption to be made because of the predictability of isotopic relationships between consumers and their food (Jardine *et al.,* 2003; Iitembu, 2014). In particular, stable isotopes of carbon and nitrogen are increasingly used in marine ecosystems, for ecological and environmental studies. This is mainly because it has proven its usefulness as an alternate, complementary, and in some cases, better tool for food web and diet analysis in

ecosystems (Lepoint *et al.,* 2004). Stable isotopes of carbon and nitrogen are valuable primarily because of their dual use as indicators of trophic levels and sources of feeding (Dawson & Siegwolf, 2007; Iitembu, 2014).

It can not be denied that the use of SIA in animal ecology addressed most shortcomings of traditional methods such as stomach content analysis. However, it should be noted that the successful application of SIA depends mainly on the knowledge of how organisms incorporate the isotopic composition of their food into their tissues (Gannes *et al.,* 1997). Stable isotopes are able to simultaneously capture multifaceted interactions, including trophic omnivory, and to track energy or mass flow through ecological communities (Peterson & Fry 1987; Kling *et al*., 1992; Cabana & Rasmussen, 1996). SIA is primarily governed by the hypothesis that the stable isotope ratios of a prey largely influence that of its predator (Peterson & Fry 1987; Gannes *et al.,* 1997; Post, 2002a). This assumption stresses that carbon and nitrogen isotope ratios in animal tissues closely reflect those of the prey items assimilated, with a predictable enrichment of the heavier isotopes (^{13}C or^{15}N) (DeNiro & Epstein, 1978). It is possible to predict the enrichment of the heavier isotopes due to preferential metabolism of the lighter ^{12}C and ^{14}N isotopes (DeNiro & Epstein, 1978, 1981).

The carbon isotope signature of animals is close to that of their diet and reflects the values of the prey they feed on, although there is a small enrichment (McCutchan *et al*., 2003). It is because of these features that it is now possible for ecologists to reveal the primary producers at the base of a food web as well as to use stable isotope analysis to quantify food sources and energy flow in aquatic systems, by tracing the movement of

elements such as nitrogen and carbon through the food web (Moncreiff & Sullivan, 2001; Connolly *et al*., 2005; Carrasco *et al*., 2012). A common approach has been to present $\delta^{13}C$–$\delta^{15}N$ bi-plots with species, individuals, or populations (Layman *et al.*, 2007), in order to identify the relative contribution of prey items to consumers (Vander Zanden & Vadeboncoeur, 2002) using mixing models such as Stable Isotope Analysis in R (SIAR).

Examples of studies where SIAR has been employed are the examination of percentage contributions of potential prey to the diet of *Deania profundorum* (Iitembu, 2014) and a study that analysed trophic levels and foraging locations of Arctic seabirds (Moody, 2007). Some studies have used Isotope values to monitor diet quality, especially the amount of protein in the diet (Michener & Lajtha, 2007). Information of dietary overlap can also be derived from the use of SIA (Iitembu, 2014). Additionally, the analysis of different tissues allows short and long-term dietary information to be gathered, since tissues vary in their turnover rates and thus in periods of food source integration (Tieszen *et al*., 1983).

Table 1: Average abundances of stable isotopes on Earth used in ecological studies (adapted from: West *et al.*, 2006).

Element	Isotope	Average abundance (%)
Hydrogen	^{1}H	99.985
	^{2}H	0.0015
Carbon	^{12}C	98.89
	^{13}C	0.11
Nitrogen	^{14}N	99.63
	^{15}N	0.37
Oxygen	^{15}O	99.759
	^{17}O	0.037
	^{18}O	0.204
Sulphur	^{32}S	95.00
	^{33}S	0.76
	^{34}S	4.22
	^{35}S	0.014

Carbon

Carbon exists primarily as the carbon-12 isotope and accounts for 98.89% of all carbon on Earth, but a small fraction (1.11%) also exists as carbon-13 (Fig. 10) (Michener & Lajtha, 2007). The two stable carbon isotopes (^{13}C and ^{12}C) occur in the molar ratio of 1:99 in the atmosphere. Carbon isotopes can be used to study and reconstruct diet

because of differential fractionation, between certain plant groups, of atmospheric carbon dioxide during photosynthesis. These different plants fix carbon differently due to their physiology leading to them having different $\delta^{13}C$ signatures. The carbon isotope signature of animals is as almost similar to that of their diet and reflects the values of the prey they feed on, although there is a small enrichment (McCutchan *et al*., 2003). Carbon isotope ratios can be used as tracers of biological energy flow to upper trophic level consumers if carbon sources have contrasting values.

Carbon source differs for organisms mainly because of physiological processes that modify ^{13}C, another source of variation in plant (whether aquatic plants or terrestrial plants) ^{13}C is the source of CO2 that is used in the photosynthesis process (Michener & Lajtha, 2007) (whether; it's an aquatic or terrestrial source). Even for aquatic trophic system, carbon ration in tissue of different organisms differ largely because $\delta^{13}C$ organisms in a marine environments are influenced by the phytoplankton and marine algae at the base of the food web (Kurle, Sinclair, Edwards & Gudmundson, 2011). Plants with C3 photosynthetic pathway generally contain proportionally less ^{13}C than the air (Farquhar, Ehleringer, & Hubick, 1989). These differences are also reflected in the consumers of these primary produces and the consumers of the secondary consumers until the final predators of such a food web. Studies have indicated that marine carbon source signatures are enriched with ^{13}C compared to terrestrial ones (Fry, 2006). Furthermore, in aquatic systems there exist differences in $\delta^{13}C$ between nearshore and offshore waters, where nearshore waters are more enriched in ^{13}C (Miller, Brodeur, & Rau, 2008). This is useful in studies of migratory organisms, since differences in isotope

ratios can be used to estimate food ingestion from the different regions that a migratory organism covers (Fry & Sherr, 1984; Schell *et al*., 1989). Vander Zanden, Hulshof, Ridgway, & Rasmussen (1998) and Post (2002a) reported that tissues of consumers are enriched by 1‰ per trophic level as one moves further along the food web. Stable carbon isotopes ($\delta\ ^{13}C$) are ideal for tracing origins of and sources of primary production (Schukat *et al.*, 2013) and energy flow in aquatic systems, identifying animal movement patterns, because they are known to fractionate little between energy transfers (Peterson & Fry 1987; Carrasco *et al*., 2012).

The amount of carbon isotope values varies in different organisms, for instance, it is reported that $\delta^{13}C$ values in aquatic plants ranges from -8 to -30‰ (Rundel & Ehleringer, 1989). Furthermore stable carbon isotopes do not only act as a strong indicator of an animal's habitat but are used as key elements in studies that estimate animal's foraging regions (Rubenstein & Hobson, 2004). In addition, $\delta\ ^{13}C$ can serve as an important environmental indicator of change (Dawson & Siegwolf, 2007).

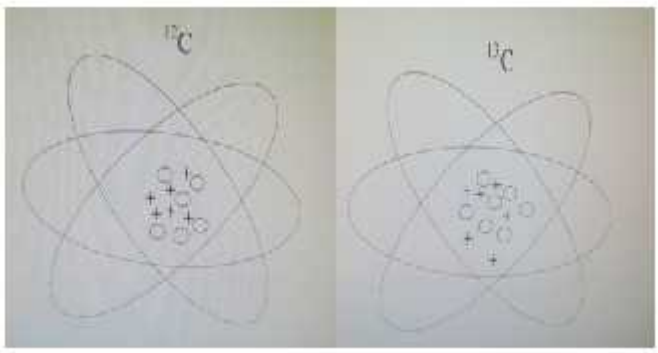

Figure 10: Generalized structure of ^{12}C (left) and ^{13}C (right), the two most frequent stable isotopes of carbon, with (+) symbols indicating protons, which are positively charged and open circles indicate neutrons (neutral) (Michener & Lajtha, 2007).

Nitrogen

Nitrogen has two stable isotopes: ^{15}N and ^{14}N (Fig. 11). ^{14}N isotope is the most abundant form of nitrogen in the air, contribution 99.64% to the total nitrogen, while ^{15}N makes up the remainder (0.36%) (Michener & Lajtha, 2007). Although ecological research has made more and more use of nitrogen stable isotopes at natural abundance levels as early as in the 1950s (Rundel & Ehleringer, 1989; Dawson & Siegwolf, 2007), the marine nitrogen cycle remains complex and still not well understood (Codispoti, 1995). Unlike carbon isotopes, nitrogen isotopes are fractionated a great deal along the trophic chains; this makes them less convenient but allows their use as an integral index of numerous ecological processes (Robinson, 2001). ^{15}N values range from -20 to +20 ‰ (Rundel & Ehleringer, 1989). Compared to carbon, which has multiple macromolecular dietary sources, nitrogen in animal protein is supplied almost entirely by dietary protein (Michener & Lajtha, 2007). Thus it can be used to determine trophic levels.

The nitrogen pools of plants and animals are enriched in ^{15}N relative to their food, with top predators having the highest concentrations of this stable isotope, in laboratory experiments (Cabana & Rasmussen, 1996). At a broad-spectrum, animals are more enriched in ^{15}N than plants and at each trophic level the consumers are generally 3.4 $^{0}/_{00}$ more enriched than the foods they consume (Minagawa & Wada, 1984; Dawson & Siegwolf, 2007). Atmospheric nitrogen (N_2) is the reference standard for nitrogen isotopic analyses and has a $\delta^{15}N$ value defined as 0 ‰. Where atmospheric nitrogen is the reference material, in animals the $\delta^{15}N$ relative to their diet is on average +3.4 $^{0}/_{00}$ for a wide variety of animal taxa. This makes $\delta^{15}N$ generally applicable for use in

determination of trophic levels (Michener & Lajtha, 2007). A positive correlation between the ^{15}N and animal position in the food chain is apparent in natural ecosystems and most laboratory experiments (Tiunov, 2007). Therefore Nitrogen isotopes are applied as a time-integrated measure of variation of trophic level among populations of the same consumer species (Cabana & Rasmussen, 1996).

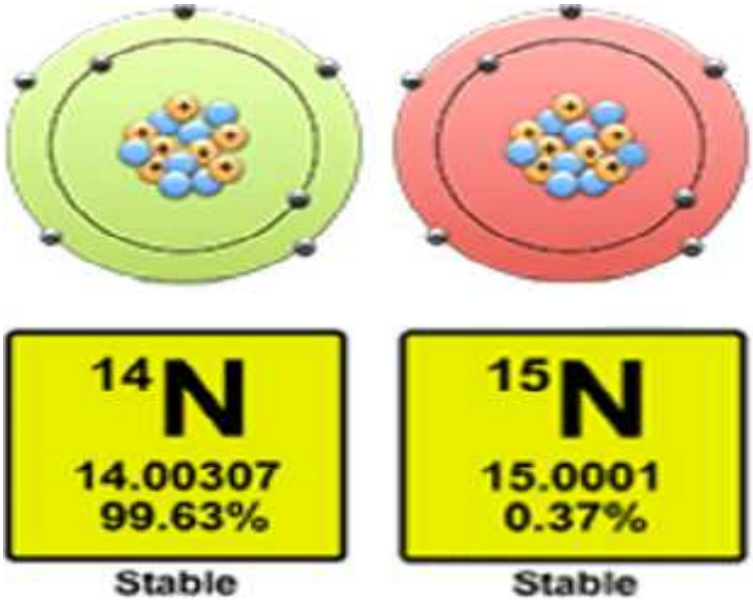

Figure 11: Generalized structure of the two regular stable isotopes of nitrogen, 14N and ^{15}N with blue circles neutrons protons, yellow circles indicate protons, grey circles indicating electrons (source: Wikipedia, 2014).

2.5.9 Inference on trophic niche from the use of stable isotopes

Food consumed by an organism may describe the trophic niche occupied by such an organism (Mohanraj & Prabhu, 2012). The trophic niche represents the overall trophic role of that species or simply the sum of all the trophic interactions that connect it to other species in an ecosystem (Leibold, 1995). Since stable isotope ratios in an organism's tissues draw from all trophic pathways ending in that individual, they can be used as one means to depict the trophic niche of that organism (Layman *et al.*, 2007). Dawson and Siegwolf (2007) elaborated the niche complementarity hypothesis, whereby they stated that plant species in an ecosystem occupy distinct ecological niches and use resources in a complementary manner, so that increasing numbers of species result in more effective resource exploitation, leading in turn to enhanced ecosystem functions. A trophic niche of given organisms may or may not overlap depending on a species' carbon and nitrogen values. Isotopic niches of two hake and three shark species in the Benguela ecosystem were compared through stable isotope analysis, since isotopic niche width show positive relationship with diet breadth of species and can be used to make inferences on their population trophic niche width (Iitembu, 2014).

2.5.10 Advantages and cautions for the use of stable isotope analysis

The use of isotopes in ecological research has become valuable (Michener & Lajtha, 2007; Iitembu, 2014), and this might address some of the short comings presented by the non-isotope analysis methods in trophic studies. Stable isotope analysis is easy to carry out; this is especially an advantage over methods that study species that are difficult to

study in the field due to the nature of their behaviour or location (Michener & Lajtha, 2007; Bodey *et al*., 2011; Iitembu, 2014). SIA also requires less man-power and is inexpensive (Bodey *et al*., 2011). Another advantage of SIA is that it is not time consuming, about 200 samples can be analysed per day, althought this depends on the nature of sample to be analysed (Michener & Lajtha, 2007). A small piece of tissue is sufficient for analysis and yield detailed trophic information. SIA is therefore an ideal and effective tool that yields reliable results. Stable isotopes are safe to handle. Perhaps the main advantage to using stable isotope analysis as opposed to stomach content observations is that no matter what the status is of the animal's stomach (empty or full), the isotope tracers in the tissues will give us an understanding of its trophic position, diet composition and food source.

However, Gannes *et al*. (1997) cautions that although the ease of stable isotope analyses makes it an attractive technique in ecological studies, care must be taken in interpretation of the results. An isotopic ratio of an organism is usually understood to represent its diet, but it should be noted that this isotopic ratio is also time specific, representing an average ratio related to tissue turnover rate and the life of the organism (Gannes *et al*., 1997). In addition, other factors such as population variability, environmental influences, and differences in individual behaviours as well as growth may affect interpretation of stable isotope results (Fulford, 2013). Moreover, trophic relationships can be difficult to resolve where there seem to be no clear isotopic difference in the possible food sources, or where an organism has a variety of potential food sources (Peterson, 1999). Although stable isotope analysis is an ideal and valuable

method in understanding trophic interactions, it does not yield much information as far as dietary preferences are concerned (Carrasco *et al*., 2012).

Chapter 3

Materials and Methods

3.1 Study area

This study was conducted in the northern Benguela upwelling system off the coast of Namibia (Fig. 12). The area is about 1500 km long and extends from the Orange River (29°S) to the Cunene River (17°S), the only perennial rivers on the Namibian coastline. The coastal plain is 100 to 200 km wide and is hyper-arid, receiving a mean rainfall of less than 100 mm per annum. The shelf area from the shore to a depth of 200 m is approximately 110 00 km^2, and about double as much to 1 000 m depth. The shelf is widest off the Orange River and off Walvis Bay, and narrowest off the Cunene River to Cape Frio (Bianchi *et al.,* 1999). This is a region of cool up-welled coastal waters found approximately between 15°S and 35°S and is generated by the intense equator-ward wind stress pattern of the Benguela system (Bianchi *et al.,* 1999). Off Namibia, strong upwelling is particularly experienced during the cooler months, which reinforces the seasonal effect and causes a definite temperature cycle (Gordoa *et al*., 2000). The major center of upwelling off is from Luderitz to the Orange River and is believed to effectively divide the Benguela system into two regions, acting as an environmental barrier for some key species. Upwelling cells of lesser intensity also occur further north, the most notable being off Cape Frio.

Namibia's 200 nautical miles Exclusive Economic Zone (EEZ)'s contain about 20 different species consisting primarily of small pelagic species (*E. encrasicolus* and *S. sagax* and juvenile *T. capensis*) and *Jasus lalandii* along the shallower onshore waters on the continental shelf, as well as large pelagic species including adult horse mackerel, demersal (hake) and other deep – sea species (*L. vomerinus*, *Austroglossus microlepis* and *Chaceon maritae*) in the waters further offshore.

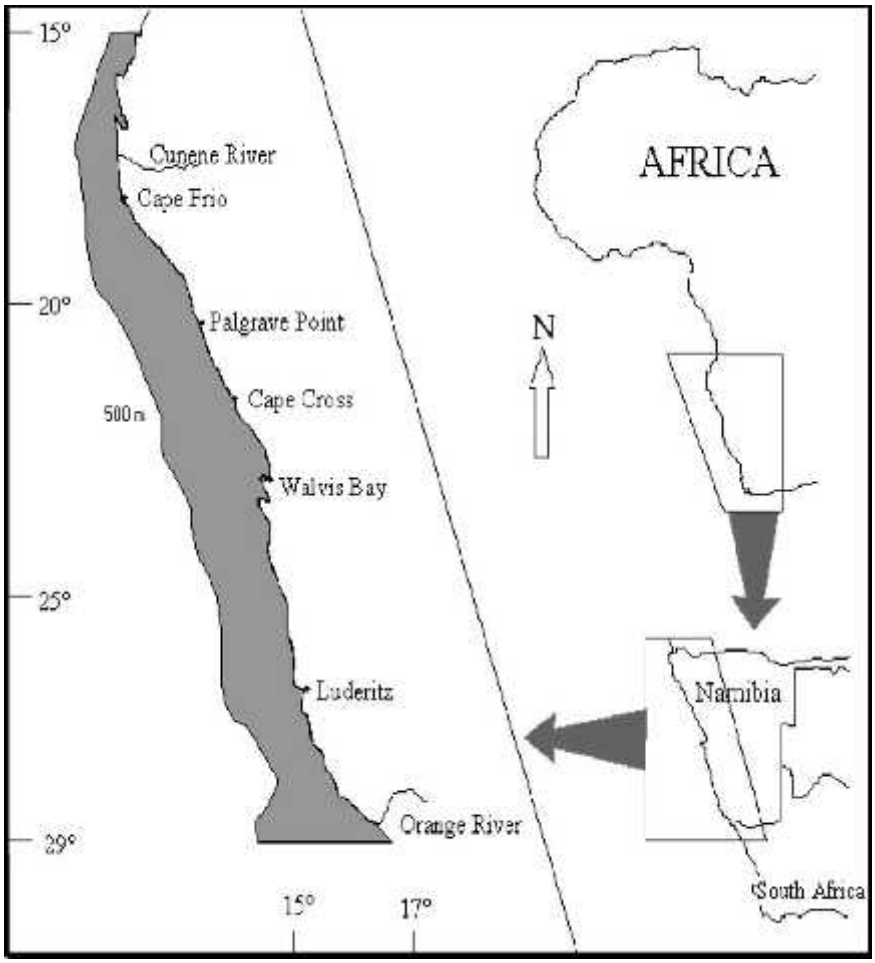

Figure 12: Study area of the northern Benguela ecosystem on the Namibian coast showing the 500 m depth contour (Heymans, Shannon & Jarre, 2004)

3.2 Sampling

3.2.1 Sampling procedure

Hake annual biomass survey

Sampling was done on board the F.V. Blue Sea I during the annual hake (*M. capensis* and *M. paradoxus*) biomass surveys conducted by the Ministry of Fisheries and Marine Resources which takes place in January and February yearly. The survey had a systematic transect design. Transects run perpendicular to the Namibian coastline, and are about 20-25 NM apart, with transect lengths ranging from 20 to 80 NM. Sampling was done using a *Gisund Super two-panel* bottom trawl with head length 31 m, footrope 47 m and the vertical net opening 4.2 to 4.5 m as described in Huse *et al.* (1997). The distance between the wings during towing was about 18-21 m. The depth of sampling tows was 90 - 600 m and trawling was done at an average trawling speed of 3 knots and average trawling time of 30 minutes. All trawl hauls were monitored by SCANMAR trawl sensors and trawl depth, bottom temperature, catch sensors, headline height and the distance between the doors were used to determine the vertical opening of the net, clearance from the bottom and the distance between the doors during trawling.

Horse mackerel biomass survey

The horse mackerel biomass survey was done on board the R.V. Welwitchia. This survey takes place in February yearly. The survey covers the area from 17°15’ to 25° 00’ S and a depth distribution from the coast to the offshore limit of the stock, which was about 500 m bottom depth south of approximately 18°33’ S and 2000 m north of this

latitude. The survey area is divided into two broad regions, inshore and offshore, separated by the 200 m depth contour. Each of the two regions are subdivided into three discrete strata, representing areas of low, medium and high densities as previously observed in the southern, central and northern regions of the Benguela system. The trawling time was generally 31 minutes on average at a trawling speed of about 4.14 knots on average. Mid-water trawl and a bottom trawl gear were used to identify selected acoustic targets observed on the echograms. Horse mackerel survey procedures standards described in D'Almeida *et al*. (2001), Kanandjembo *et al*. (2004) and Uanivi & Van der Plas (2013).

Monkfish biomass survey

The Monkfish (*L. vomerinus*) biomass surveys took place onboard R.V Welwitchia mostly during the months of November 2012 and 2013. This survey covered a total of 91 stations, covering the bottom depth between 100 to 700 m. The distance between $17^0$15'S and 30^0S of the coast was divided into 40 equal intervals, while the east-west direction was divided in 19 NM intervals. The survey area was defined by a polygon of the assumed distribution of monkfish, which was then sub-divided into smaller cells. Trawling time averaged 30 minutes at a depth of 650 to 700 m. The survey design followed the optimized geo-statistical stratified random design described in Schneider and Johnsen (2000) and all other Monkfish Biomass Survey Cruise Reports of the Ministry of Fisheries and Marine Resources.

3.2. 2 Sampling Techniques

Specimens of non-commercial species including *E. whiteheadi* (Fig. 3), Myctophidae (*S. boops, L. australis, L. hectoris, D. meadi* and *D. hudsoni*) (Fig. 4) and *Sepia* spp. (*S. australis* and *S. elegans*) (Fig.5), were collected at 51 stations from the Orange River (29°S) to the Cunene River (17°S), (Fig. 13). The exact amount of specimens collected at a particular station depended on the total amount of specimens of these species in the catch. Sampling for all fish was opportunistic, with the general aim of obtaining a wide size distribution of each of the eight prey species to examine their trophic relationships. The probability of any specimen to be collected from its population was completely unknown. At each sampling station the entire catch was brought on deck to be sorted manually into species and, if present, individual specimens of *E. whiteheadi*, Myctophidae and *Sepia* spp. were collected. If there were less than eight individuals of a species all individuals were sampled and if there were more than eight individuals sampling were done to ensure that all size classes are covered and a maximum of eight specimens were then collected. The total length (cm) and weight (g) of the collected specimens of *E. whiteheadi*, Myctophidae and *Sepia* spp. were measured and recorded. Information such as species identification, date of collection, station number and position were also recorded. The samples were placed in ziplock bags and frozen at about -20°C on the vessel until the end of the survey for approximately three weeks. Sampling was done during the period of September 2012 to March 2014.

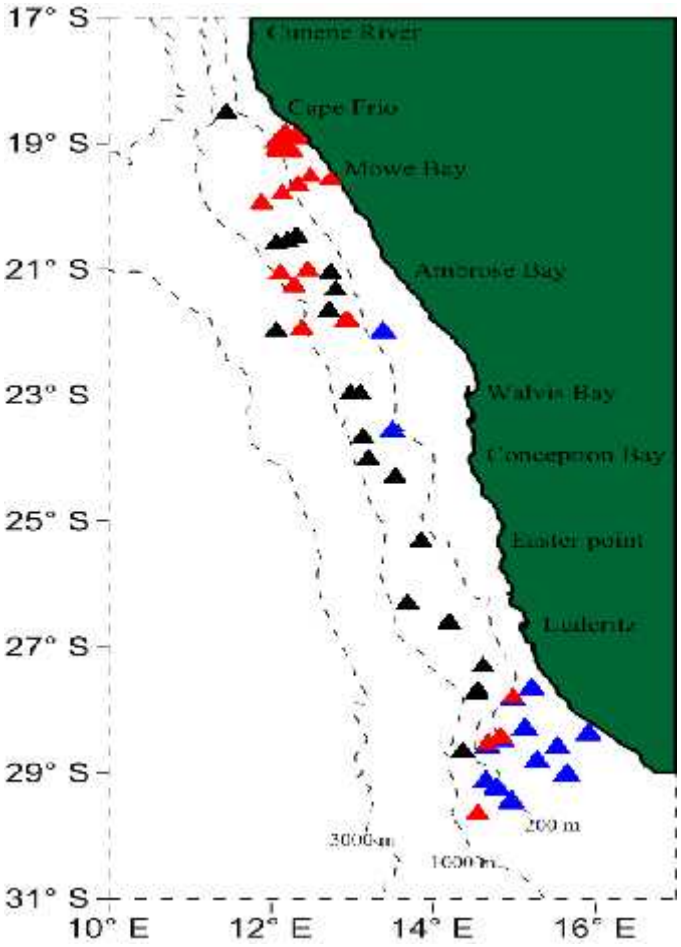

Figure 13: Geographical positions where samples for *Etrumeus whiteheadi* (red triangle), myctophids (black triangle) and *Sepia* spp. (blue triangle) were collected off Namibia. Depth contours represent 200, 1000 and 3000 m isobaths.

3.2.3 Collection of predator samples

A total of 156 samples of predators (*M. capensis* = 25, *M. paradoxus* = 47, *L. vomerinus* = 23 and *T. capensis* = 61) were collected for this study. The geographical positions from which samples were obtained are shown in Figure 14. All predators (*M. paradoxus*,

M. capensis, L. vomerinus and *T. capensis*) samples were collected in the same manner as those of *E. whiteheadi*, Myctophidae and *Sepia* spp. and from the three annual biomas surveys; hake annual biomass survey, horse mackerel biomass survey and monkfish biomass survey.

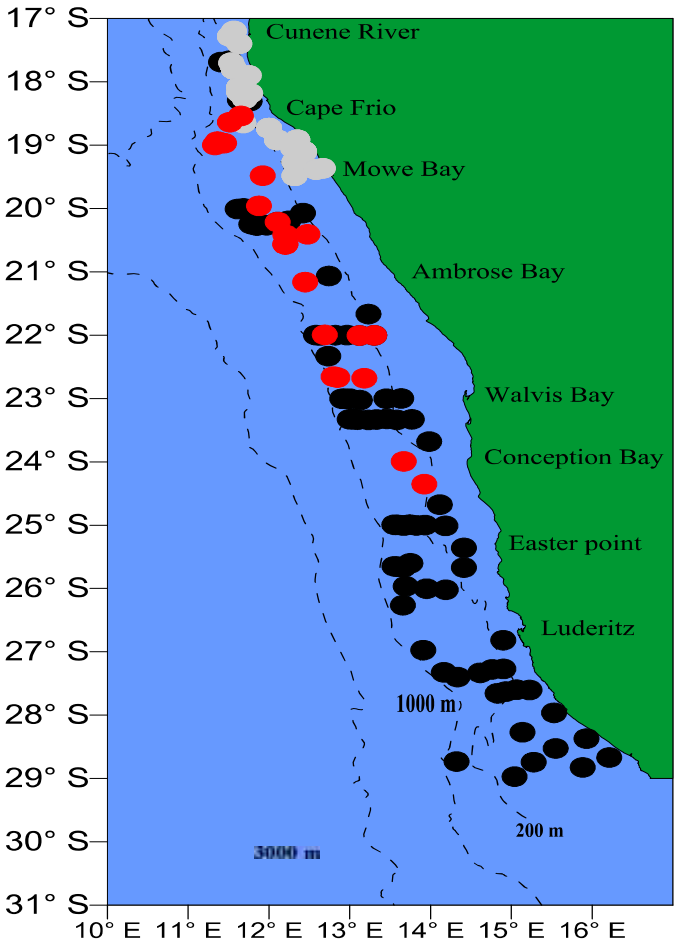

Figure 14: Geographical positions where samples for *Merluccius capensis, Merluccius paradoxus* (black circles) *Lophius vomerinus* (red circles) and *Trachurus capensis* (grey circles) were collected off Namibia. Depth contours represent 200, 1000 and 3000 m isobaths.

3.2.4 Preparations of samples

Samples collected at sea were brought on land and processed at the National Marine Information and Research Centre (NatMRIC) in Swakopmund for laboratory preparation. The scales and skin of each fish were carefully removed (Fig. 15). A small piece of white muscle approximately 1cm in length and 1 cm wide was cut from the anterior-dorsal region of each fish individual and thereafter placed in an aluminum foil and labeled. The tissues in the labeled aluminum foil were dried in a drying oven for 48h, at 60 °C. A mortar and pestle were used to grind the dried tissues into a fine powder which was placed into labeled polyethylene vials for stable isotope analysis at IsoEnvironmental CC Laboratory (Rhodes University, Grahmstown, South Africa). The remaining whole fish was stored in labeled Ziploc bags and frozen at -20^{o} C atNatMIRC.

Figure 15: An indication of how a tissue for isotope analysis was obtained from the anterior-dorsal region of a fish

3.3 Stable isotope analysis

At Rhodes University, powdered samples were further grinded with an electronic grinder and thereafter samples were placed into tubes, together with metallic balls that further pulverize the sample into a fine powder. Sub-sample aliquots were subsequently weighed out from the samples to a specific weight. The exact amount of material is dependent on the sensitivity of the electro mass spectrometer used in relation to the species to be analyzed. The sample weight for *Sepia* spp. ranged between 1.0 – 1.2 mg. While those of *E. whiteheadi*, and Myctophidae ranged between 0.99 – 1.28 mg and between 1.0 – 2.24 mg, respectively. The weighed powdered sub-samples were placed in small tin capsules of about 8 mm x 5 mm. Tin capsules are ideal because they burn out completely compared to capsules of other elements. The capsules were folded as much as possible and squashed to stop the air from entering. The standards were also weighed. The standards used in the mass spectrometer were those of OAS (Organic Analytical Standard). These standards are made up of a homogenous batch of protein known as Casien, extracted from animals.

This is a routine working microanalytical standard protein used for the determination of the Carbon (^{13}C), Nitrogen (^{15}N) isotopes. Samples in folded tin capsules together with standards were then loaded; one at a time, into the column of the mass spectrometer with forceps. The computer connected to the EMS was used to monitor the precence of gases so that the old oxygen coul be flushed out and to ensure that all gases were clean.

An autosampler placed the samples into a furnace of the gas combustor at about 1000°C and combusted under high vacuum in a Costech Elemental Analyzer attached to a Thermo-Finnigan delta plus Isotope Ratio electro mass Spectrometer.

The gases were passed to a Delta Plus XP IRMS (isotope ratio mass spectrometer), via a Conflo III gas control unit (Thermo Finnigan). It takes a minute for combustion of each sample. Samples need to be combusted in order to be converted from solid state to a gaseous state, since samples can only be analyzed in that state and also to eliminate other elements that are neither nitrogen nor carbon.The ion current creates voltage as it flows, which is then used as the output from the mass spectrometer and fed to the computer. The combusting process is monitored with a computer connected to the EMS. This computer is used to monitor precence of gases such as atmospheric oxygen, nitrogen and carbon. The isotopic values of the resultant N_2 and CO_2 gases from the EMS, were expressed in delta scale (δ) notation as parts per thousand (‰). The δ notation indicates the differences of the isotopic composition of a sample from an international accepted standard based on the following equation adopted from Hobson (1993):

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad \text{(Equation 1)}$$

Where *X* is the heavy isotope in question (^{13}C, ^{15}N), R is the ratio of the heavy to the lighter isotope of the element (^{15}N /^{14}N, $^{13}C/^{12}C$), R_{sample} and $R_{standard}$ are the isotopic ratios of the sample and standard atmospheric (AIR) nitrogen for $\delta^{15}N$, ^{13}C - casein, respectively. The measurements error (SE) at IsoEnvironmental CC Laboratory was of ±0.06 ‰ for $\delta^{13}C$ and ±0.12 ‰ for $\delta^{15}N$. Samples were not lipid extracted as

recommended by some studies (McConnaughy & McRoy, 1979; Post *et al*., 2007), however, the $\delta^{13}C$ values were mathematically corrected for variations in lipid a mathematical. Physical lipid extraction was not done mainly because it has negative effects on the samples as it causes fractionation in $\delta^{15}N$ (Sweeting, Polunin & Jenning, 2006; Post *et al.,* 2007) and it is not a prerequest prior to sample analysis.

3.4 Data Analysis

3.4.1 Trophic level calculations

To calculate the relative trophic level (TL) of *Etrumeus whiteheadi*, Myctophidae (*S. boops, L. australis, L. hectoris, D. meadi* and *D. hudsoni*) and *Sepia* spp. (*S. australis* and *S. elegans*), the following equation was used:

$$Trophic\ level = [(\delta^{15}Nconsumer - \delta^{15}Nbase)/\Delta\delta^{15}N] + 2.0 \quad (Equation\ 2)$$

where $\delta^{15}N$consumer is the signature of the consumer (example for *Sepia* spp.), $\delta^{15}N$base is the baseline value of the food web, $\delta^{15}N$ is the trophic enrichment factor, which was set at 3.4 ‰ in $\delta^{15}N$ per trophic level (Minagawa & Wada 1984; Post, 2002b), and the value 2.0 indicates the trophic level of the organism used to establish the $\delta^{15}N$base. An isotopic baseline is a prerequest in calculations of distinct trophic levels (Schukat *et al*., 2013). No baseline organisms such as mussels or zooplankton were collected, and the ones used in this study were collected by Iitembu (2014). A 9.2 ‰ was used as a mean $\delta^{15}N$base and was obtained from two mytilid bivalve species (*Choromytilus meridionalis* and *Mytilis galloprovincialis*). For each of the species analyzed, a mean $\delta^{15}N$, $\delta^{13}C$ and C:N and their standard deviation (SD) were calculated

(Table 2).All statistical tests were performed using R (R Core Team 2009), Vienna, Austria).

3.4.2 Trophic relationship of the prey species looking at $\delta^{15}N$ and $\delta^{13}C$ values

Data was examined for normality and homogeneity of variance using the Shapiro–Wilk and Levene tests, respectively. Violations of normality and homogeneity were addressed using log10 transformation of the data. The log transformed data was used for further analysis. All C:N values greater than 3.5 (the minimum limit for lipid extraction or correction stipulated in Post *et al.* (2007b) were mathematically corrected for variations in lipid using the normalization equation below from Post *et al.* (2007b):

$$\delta 13Cnormalized = \delta 13Cuntreated - 3.32 + 0.99 \times C{:}N$$

where δ13C untreated is the δ13C of non-lipid extracted tissue, *C:N* is the mass ratio of carbon and nitrogen .

Variation in stable isotope of $\delta^{15}N$ and $\delta^{13}C$ values among the species (*E. whiteheadi*, Myctophidae (*S. boops, L. australis, L. hectoris, D. meadi* and *D. hudsoni*) and *Sepia* spp. (*S. australis* and *S. elegans*) were compared and tested for significance with Analysis of variance (ANOVA). All post-hoc comparisons were done with a Tukey HSD (Tukey Honest Significant Difference) test. Statistical tests were performed at a significance level (alpha) of 0.05. All statistical tests were performed using R (R Core Team 2009), Vienna, Austria).

3.4.3 Trophic niche calculations

Calculation of isotope-based metrics such as (a) ^{15}N range, indicating trophic diversity, (b) $\delta^{13}C$ range representing the niche diversification at the base of a food web and (c) total area (TA) of the convex hull standard ellipse areas (SEA_B) for each species, were done.

(a) $\delta^{15}N$ Range (NR): is the distance between the two species with the most enriched and most depleted $\delta^{15}N$. NR values were calculated based on the following equation:

(*maximum$\delta^{15}N$ - minimum $\delta^{15}N$) (Equation 3)* (Layman *et al.,* 2007) (Table 2 and 3).

(b) $\delta^{13}C$ range (CR): is the distance between the two species with the most enriched and most depleted $\delta^{13}C$ values. CR values were calculated based on the following equation: (*maximum$\delta^{13}C$ - minimum $\delta^{13}C$*) *(Equation 4)* (Layman *et al.,* 2007) (Tables 2 and 3).

(c) Total area (TA): Convex hull area representing total amount of niche area occupied by species, giving an indication of niche width was done following procedures in Layman *et al.,* 2007 and indicated in $\delta^{13}C$– $\delta^{15}N$ bi-plot space (Figs. 16 and 18). TA is influenced by species with extreme positions on either the $\delta^{13}C$ or $\delta^{15}N$ axis, or both, and thus typically will be correlated to some degree with these two metrics. Details on calculating convex hull area and volumes are described in Cornwell, Schwilk & Ackerly (2006).

d) Bayesian standard ellipse areas (SEA_B) were estimated for each species (Fig. 17 and 18) (Jackson *et al.,* 2011) using the new metrics, SIBER (Stable Isotope Bayesian

Ellipses in R) to directly compare isotopic niches across species. Bayesian estimate of the standard ellipse (SEA_B) and its standard ellipse area (SEAc) for all species was produced.

To test whether one Species SEA is smaller than the other species, the proportion of ellipses of all species were calculated. SEAc, which is the standard ellipse area size (Jackson *et al.* 2011), was calculated also in R (R Core Team 2009), Vienna, Austria). The Trophic niche comparisons of the prey species is depicted by convex hull and SEAc which nullifies the bias introduced by sample size (Jackson *et al.*, 2011). Details on calculating convex hull area and volumes are described in Cornwell *et al.* (2006).

3.4.4 Dietary contributions of different prey species to the diets of commercial species

An isotope mixing model was used to determine the likely contributions of different preys to the predators' diets.

A Bayesian stable-isotope mixing model, termed Stable Isotope Analysis in R (SIAR) (Parnell, Inger, Bearhop & Jackson, 2010), was used to obtain the feasible contributions of the different species to the isotopic signatures of the three commercial species (*M. paradoxus*, *M. capensis* and *T. capensis*). This model basically estimates the proportional contributions of sources to a mixture (Phillips, 2012). Isotopic signatures and fractionation as well as the uncertainties of these values were incorporated in the model (Parnell *et al.* 2010). 3.2 ±1.28‰ was used as the fractionation factors for $\delta^{15}N$

(Sweeting, Barry, Polunin, & Jennings, 2007) and 1.56±1.10‰ for $\delta^{13}C$ (Sweeting *et al.*, 2007). It is vital to make corrections to the diet and consumer isotope values for this systematic bias before the mixing analysis is performed (Phillips, 2012).

Chapter 4

Results

4.1 Species isotopic characteristics

The results failed tests for normality and homogeneity of variance P<0.05 for δ15N and δ13C'. However, the length data was both homogeneous and normally distributed. The number of specimens for each species ranged from 7 to 117 (*E. whiteheadi* (n = 117), Myctophidae (n = 146), *Sepia* spp. (n = 71). The sizes of samples varied from the smallest *L. hectoris* of 4.5 to the largest *S. boops* of 23 cm (Table 2).

$\delta^{15}N$ for all prey species ranged from 10.94‰ to 12.61 (Table 2). Most prey species displayed enriched $\delta^{15}N$ values and relative high trophic levels compared to the baseline of mytilid bivalve species from Iitembu (2014) ($\delta^{15}N$ = 9.2‰). Among the prey species analysed, *S. boops* had the most depleted $\delta^{15}N$ (10.94 ‰), while *L. hectoris* had the most enriched $\delta^{15}N$ values (12.61‰). All species (*E. whiteheadi, S. boops, L. australis, L. hectoris, D. meadi, D. hudsoni, S. australis* and *S. elegans*) had $\delta^{15}N$ above zooplankton and nekton prey (Table 2) , and had trophic levels above 2.0 (Table 3).

The δ13C' values for most of the prey species ranged from -17.72 ‰ to -16.86‰. All species showed much depleted δ13C' values however, *D. hudsoni was* the most depleted (most negative) values (δ13C' = -17.72‰) while *E. whiteheadi was* the least (least negative) depleted values (δ13C' = -16.86 ‰). The mean ratio of carbon-to-nitrogen (C:N) ratio was lowest for *E. whiteheadi* (3.50) and highest for *D. hudsoni* (5.33). Carbon-to-nitrogen (C:N) for all analysed prey species were greater than 3.5 (Table 2).

Table 2: Stable isotope measurements [‰ (standard deviation)] of *Etrumeus whiteheadi,* Myctophidae (*Symbolophorus boops, Lampanyctus australis, Lampanyctodes hectoris, Diaphus meadi* and *Diaphus hudsoni*) and *Sepia* spp. (*Sepia australis* and *Sepia elegans*) with their average total length [L (cm)], number of samples (n), average normalized Nitrogen values ($\delta^{15}N$) average Carbon values ($\delta^{13}C$) and carbon: nitrogen ratios (C: N). The δ13C' symbolizes the lipid-normalized values.

Species	**N**	**L (cm)**	**δ^{15N}**	**$\delta^{13}C$**	δ13C'	**C:N**
Myctophids	**146**	**7.78**	**11.26(**±1.14)	**-18.45(**±1.13)	**-17.32(**±0.82)	**4.5**
S. boops	66	8.61	10.94**(**±0.97)	-18.92**(**±1.08)	-17.44**(**±0.83)	4.13
L. australis	28	7.62	11.8**(**±1.16)	-18.24**(**±1.21)	-16.99**(**±0.79)	4.61
L. hectoris	20	6.4	12.61**(**±0.73)	-18.49**(**±0.58)	-17.24**(**±0.72)	4.61
D. meadi	21	6.91	11.73**(**±1.19)	-19.44**(**±1.31)	-17.24**(**±0.82)	4.93
D. hudsoni	11	7.28	11.12**(**±0.25)	-19.68**(**±0.65)	-17.72**(**±0.53)	5.33
***Sepia* spp.**	**71**	**7.89**	**11.74(**±0.70)	**-17.57(**±0.59)	**-17.34(**±0.73)	**3.58**
S. australis	64	7.85	11.74**(**±0.64)	-17.59**(**±0.35)	-17.36**(**±0.46)	3.58
S. elegans	7	8.28	11.75**(**±1.18)	- 17.4**(**±1.66)	-17.16**(**±0.74)	3.59
E. whiteheadi	**117**	**17.45**	**11.09(**±1.77)	**- 17.0(**±1.17)	**-16.86(**±1.15)	**3.50**

4.2 Trophic levels of prey species

Myctophids species, *Sepia* spp. and *E. whiteheadi* were trophically indistinguishable at TLs of 2.67, 2.75 and 3.00, respectively (Table 3). *Diaphus meadi, S. australis* and *S. elegans* all occupied 2.75 trophic level, *E. whiteheadi* fed at a trophic level of 2.67 (Table 3). *Lampanyctus australis* fed at a trophic level of 2.77, while *L. hectoris* of the myctophids fed at the highest trophic level compared to the other species analyzed (mean $\delta^{15}N$ of 12.61 ‰, trophic level 3.00). The lowest feeding trophic levels during this study were generally recorded for *S. boops* that fed at TL 2.51, (Table 3) compared to the other species analyzed.

Table 3: Trophic level of eight prey species

Species	**TL**
Myctophids	**2.67**
Symbolophorus boops	2.51
Lampanyctus australis	2.77
Lampanyctodes hectoris	3
Diaphus meadi	2.75
Diaphus hudsoni	2.57
***Sepia* spp.**	**2.75**
Sepia australis	2.75
sepia elegans	2.75
Etrumeus whiteheadi	**2.67**

4.3 Trophic relationship of the prey species

An analysis of variance (ANOVA) test results indicated significant variations in δ13C' ($P < 0.01$, $F = 6.16$) and $\delta^{15}N$ ($P < 0.01$, $F = 2.74$) isotope ratio among prey species analysed. Pairwise Tukey HSD post hoc test indicated that most species were not significant different from each other in term of $\delta^{15}N$ and δ13C'. However, significant differences were found between the following species: *L. hectoris* was significantly different from *D. hudsoni* ($P = 0.04$), *E. whiteheadi* ($P = 0.00$) and from *S. boops* ($P = 0.00$) in term of $\delta^{15}N$ (Table 4). *Sepia australis* was significantly different from *E. whiteheadi* ($P = 0.02$) and *S. boops* ($P = 0.01$) in term of $\delta^{15}N$ (Table 4).

Table 4: Tukey multiple comparisons of means of $\delta^{15}N$ among prey species that were significantly different from each others.

Prey species compared	$\delta^{15}N$
L. hectoris - *D. hudsoni*	P = 0.04
L. hectoris - *E. whiteheadi*	P = 0.00
L. hectoris - S. *boops*	P = 0.00
S. australis- *E. whiteheadi*	P = 0.02
S. australis- S. *boops*	P = 0.01

In terms of δ13C', *D. hudsoni* was significantly different from all the prey species analysed ($p<0.05$). *Etrumeus whiteheadi* was also significantly different from most of the prey species ($p<0.05$), with an exception of *S. australis. Sepia australis* was also

significantly different from *S. Boops* (P = 0.01), *L. hectoris* (P = 0.01) and *D.meadi* (P = 0.00), *S. elegans* was also significantly different from *D.meadi* (P = 0.04) (Table 5).

Table 5: Tukey multiple comparisons of means of δ13C' among prey species that were significantly different from each others.

Prey species compared	δ13C'
D. hudsoni- L. hectoris	P = 0.04
D. hudsoni -L. australis	P = 0.02
D. hudsoni - E. whiteheadi	P = 0.00
D. hudsoni - S. *boops*	P = 0.00
D. hudsoni- S. elegans	P = 0.00
D. hudsoni - S.australis	P = 0.00
E. whiteheadi -S.boops	P = 0.00
E. whiteheadi -L.australis	P = 0.00
E. whiteheadi -L. hectoris	P = 0.00
E. whiteheadi -S.australis	P = 0.00
E. whiteheadi - D.meadi	P = 0.00
S.australis - S.boops	P = 0.01
S.australis- L. hectoris	P = 0.01
S.australis-D.meadi	P = 0.00
S. elegans- D.meadi	P = 0.04

4.4 Trophic niche of prey species

Isotope-based population metrics indicated that the trophic niches of all different prey species overlapped (Fig. 16 and Table 6). The trophic niches of *S. boops* was however, the widest (10.86) while for *D. hudsoni* was the narrowest (1.04) (Fig. 16 and Table 6). *Symbolophorus boops* had widest δ^{15}NR (4.82) while *D. hudsoni* had lowest NR (0.76) (Table 6). The δ13CR' was lowest for *D. hudsoni* (1.74), and highest for *E. whiteheadi* (8.64) (Table 6). *Sepia elegans* had the largest SEAc (3.36), while *D. hudsoni* had the lowest (0.59) (Table 6).

The trophic niche sizes indicated by the Bayesian estimate of the standard ellipses for *E. whiteheadi, D. hudsoni, L. australis, S. boops* and *S. elegans* significantly overlap (Fig 17). The trophic niche sizes for *D. hudsoni, L. hectoris* and *S. australis* also overlap significantly (Fig. 17).

Comparisons of *E. whiteheadi*, to grouped myctophids and *Sepia* spp. indicated that the trophic niche of myctophids was the widest (20.60), while the niche for *Sepia* spp. was the smallest (9.65) (Fig. 19 and Table 7). Myctophids had widest δ^{15}N range (5.46) and δ13C' ranges (6.43), while *Sepia* spp. had the narrowest δ^{15}N range (4.06), as well as the lowest δ13C' (0.39) (Table 7). *Sepia elegans* had the lowest SEAc (1.12), myctophids had the highest (3.94) (Table 7).The trophic niche size for *E. whiteheadi* and myctophids overlap, but it does not overlap with that of *Sepia* spp. (Fig. 18). Trophic niche size indicated by the Bayesian estimate of the standard ellipse (Fig. 19) is significantly smaller for *Sepia* spp. (p = 9.65) and widest for myctophids (Table 7).

Table 6: Carbon range (δ13 C'R), nitrogen range (δ15 NR), total area of the convex hull (TA) and standard ellipse area (SEAc) of *Etrumeus whiteheadi*, *Symbolophorus boops*, *Lampanyctus australis*, *Lampanyctodes hectoris*, *Diaphus meadi*, *Diaphus hudsoni*, *Sepia australis* and *Sepia elegans* .

Species	δ15 N R	δ13 C' R	SEA	SEAc	TA
Etrumeus whiteheadi	4.73	8.64	2.50	2.52	10.80
Diaphus hudsoni	0.76	1.74	0.53	0.59	1.04
Diaphus meadi	4.27	3.57	2.26	2.38	7.74
Lampanyctus australis	3.61	3.60	3.20	3.33	9.59
Lampanyctodes hectoris	3.31	2.96	1.80	1.90	5.02
Sepia australis	3.66	2.17	0.90	0.92	5.82
Symbolophorus boops	4.82	3.66	3.66	2.15	10.86
Sepia elegans	3.39	4.91	4.91	3.36	4.17

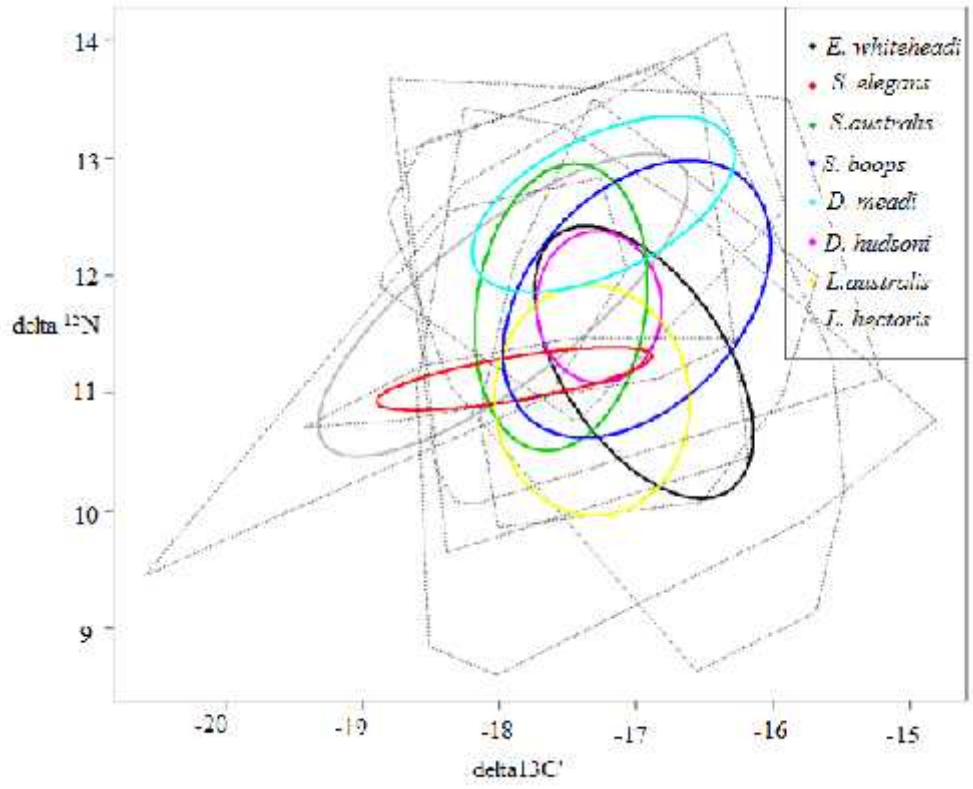

Figure 16: Trophic niche comparisons of *Etrumeus whiteheadi*, *Symbolophorus boops*, *Lampanyctus australis*, *Lampanyctodes hectoris*, *Diaphus meadi*, *Diaphus hudsoni*, *Sepia australis* and *Sepia elegans* as portrayed by convex hull (polygons) and SEAc (circle)**.**

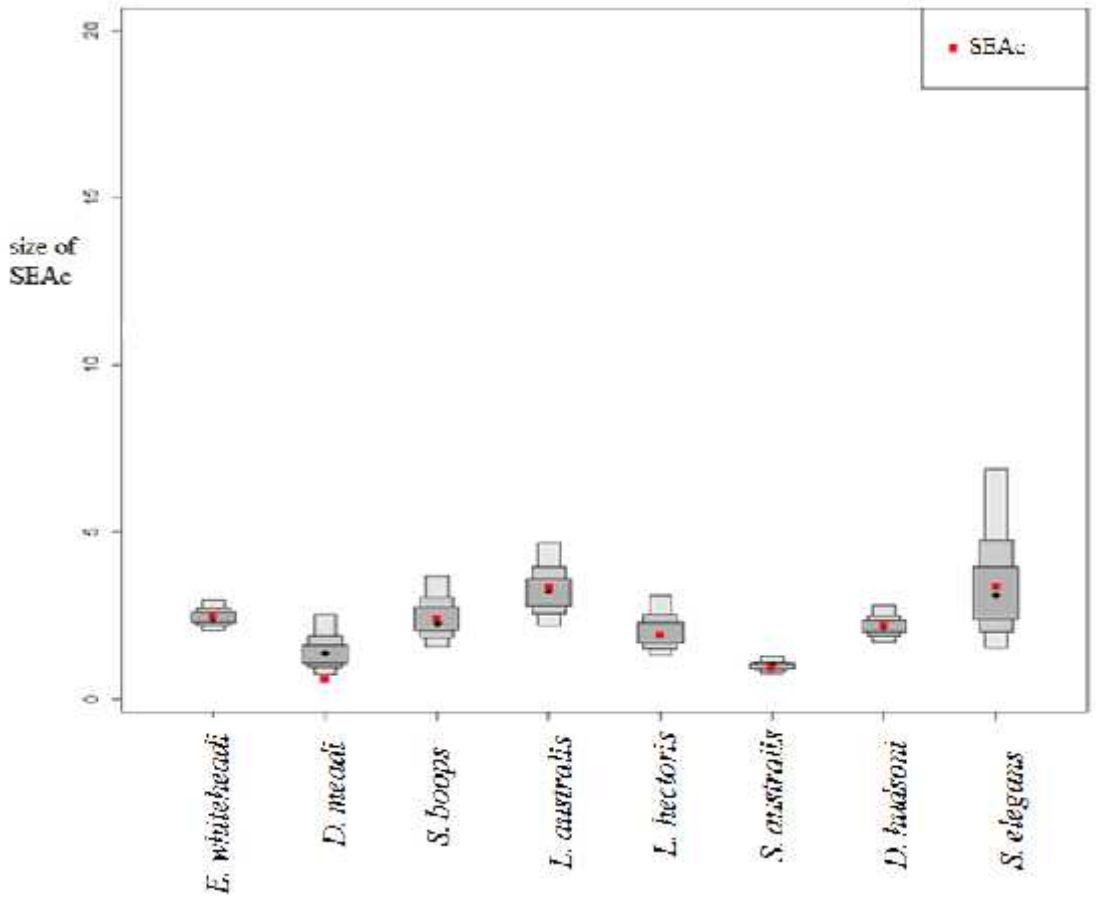

Figure 17: Bayesian estimate of the standard ellipse and its area SEA_c *Etrumeus whiteheadi*, *Symbolophorus boops*, *Lampanyctus australis*, *Lampanyctodes hectoris*, *Diaphus meadi*, *Diaphus hudsoni*, *Sepia australis* and *Sepia elegans*.

Table 7: The Carbon range (δ13 C'R), nitrogen range (δ15 NR), total area of the convex hull (TA) and standard ellipse area (SEAc) of *Etrumeus whiteheadi*, myctophids and *Sepia* spp.

Species	δ15 N R	δ13 C'R	SEA	SEAc	TA
Etrumeus whiteheadi	4.73	3.05	2.98	3.01	15.37
Myctophidae	5.46	6.43	3.91	3.94	20.60
Sepia spp.	4.06	0.39	1.11	1.12	9.65

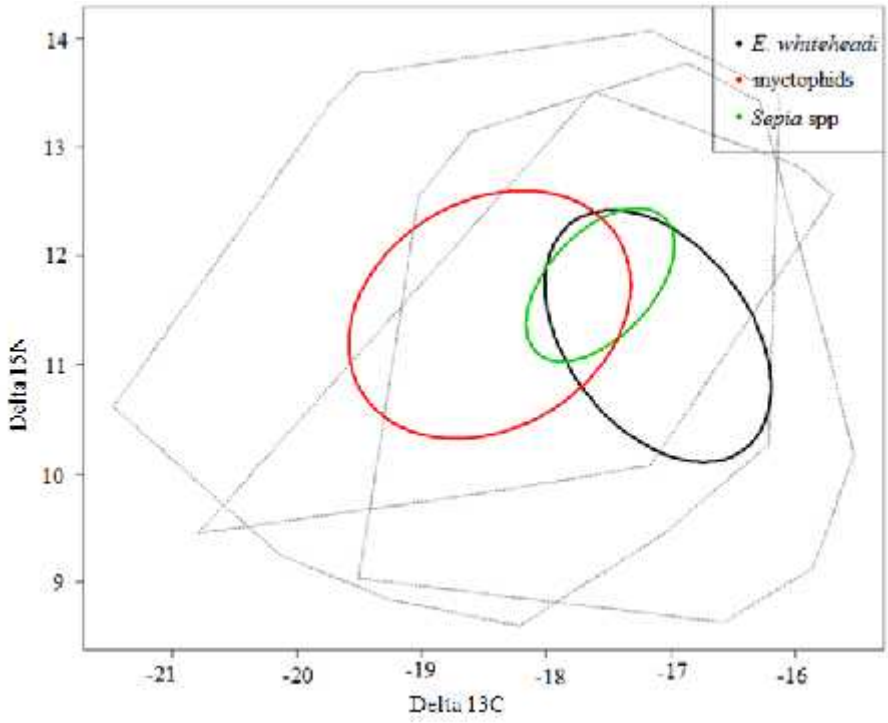

Figure 18: Trophic niche comparisons of *Etrumeus whiteheadi*, Myctophidae and *Sepia* spp. as depicted by convex hull (polygons) and SEAc (circle).

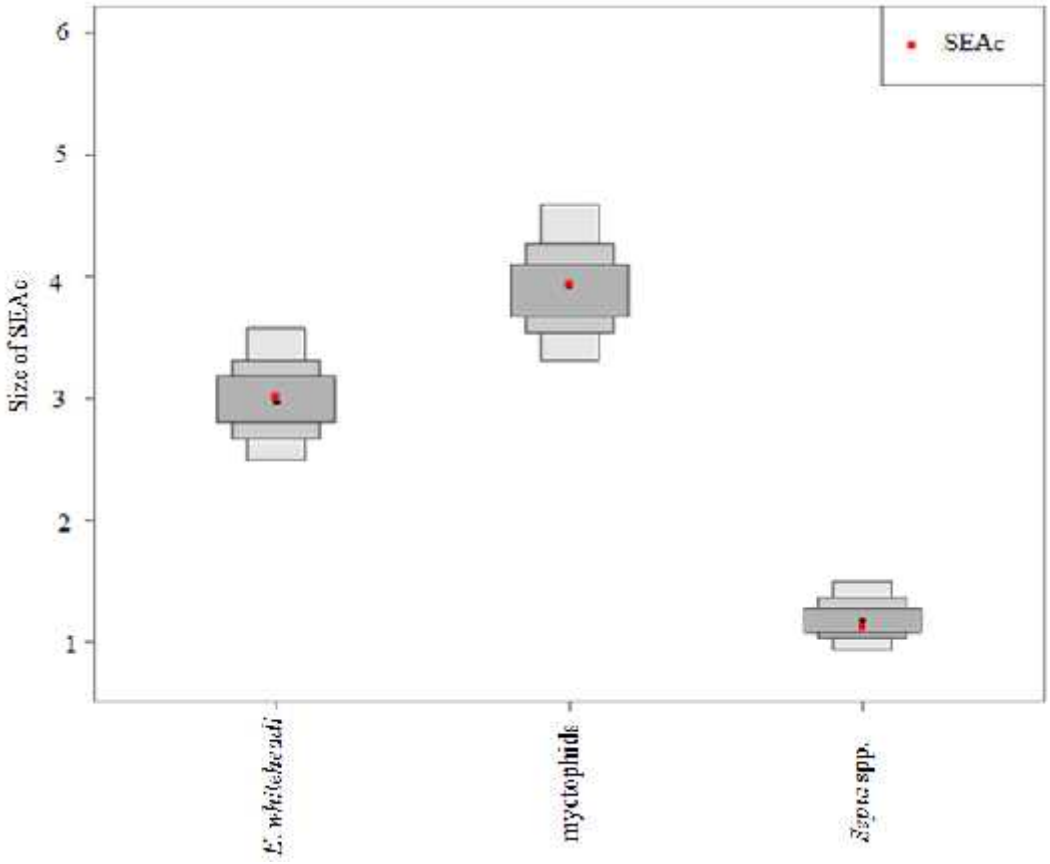

Figure 19: Bayesian estimate of the standard ellipse and its area SEAc for of *Etrumeus whitehead*, myctophids and *Sepia* spp.

4.5 Trophic niches of the predators

The trophic levels of the predator species were between 2.15 and 3.36 (Table 8). *Lophius vomerinus* fed at the highest trophic level (3.36), while *M. capensis* fed at the lowest trophic level (2.98), (Table 8). Table 8 also shows the diets (isotopic mixture) of four consumers (*M. capensis n* = 25, M. *paradoxus n* = 47, *L. vomerinus n = 23* and *T. capensis* = 61) which were analysed.

The trophic niche of *M. paradoxus* (black circle), is the smallest while that of *T. capensis* is the widest (Fig. 20 and 21). The trophic niche for *M. capensis*, M. *paradoxus* and *T. capensis* overlap (Fig. 21). *Lophius vomerinus'* trophic niche does not overlap with those of the other three predator species. *Merluccius paradoxus* and *M. capensis* occupied a relatively small area of isotopic niche space relative to *L. vomerinus* and *T. capensis* (Fig. 21).

Table 8: Stable isotope Mean (±SD) values of stable nitrogen and carbon isotope ratios of muscle tissues from consumers that were used in the SIAR model and their average C: N ratios and trophic level (TL).

Species	*N*	$\delta^{15}N$	$\delta^{13}C$	C:N	TL
M. capensis	25	9.71(±1.24)	-16.34(±0.64)	3.31	2.15
M. paradoxus	47	12.54(±1.72)	-16.90(±0.67)	3.21	2.98
L. vomerinus	23	14.91(±1.06)	-15.79(±0.90)	3.14	3.36
T. capensis	61	10.96(±1.61)	-16.32(±0.67)	3.43	2.52

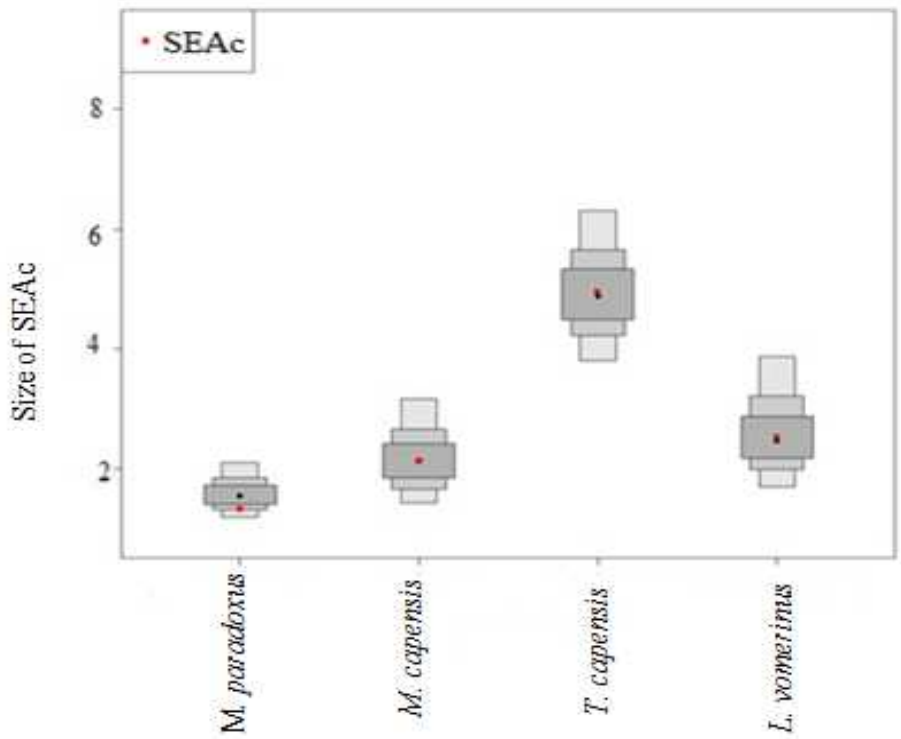

Figure 20: Bayesian estimate of the standard ellipse and its area SEAc for of *Merluccius capensis*, *Merluccius paradoxus*, *Trachurus capensis* and *Lophius vomerinus*.

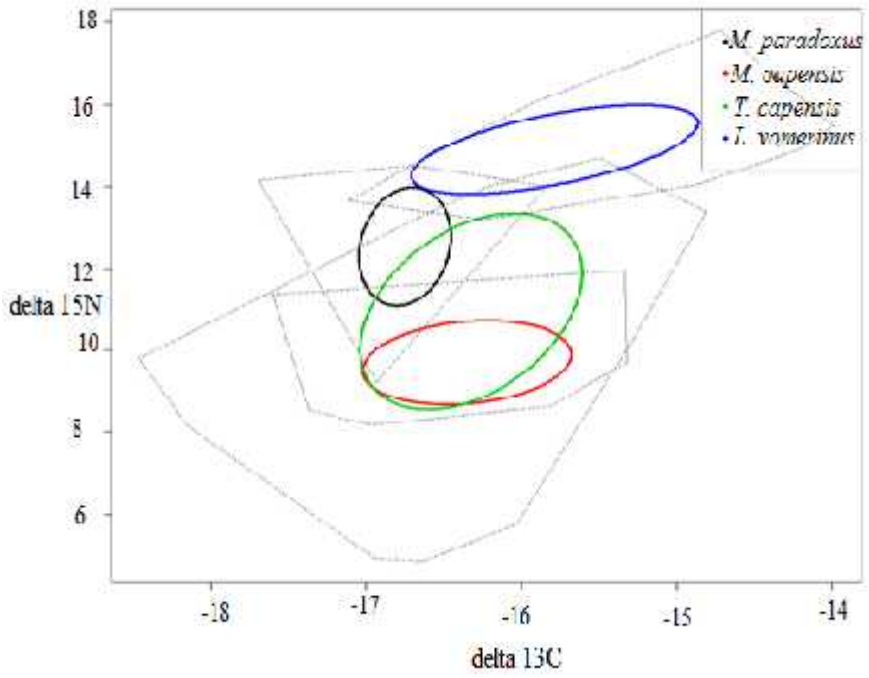

Figure 21: Trophic niche comparisons of *Merluccius capensis*, *Merluccius paradoxus*, *Lophius vomerinus* and *Trachurus capensis* as depicted by convex hull (polygons) and SEAc (circle).

4.6 Dietary contributions of different prey species to the diets of some of the commercial species.

4.6.1 Dietary contributions of different prey species to the diets of *M. capensis*

Etrumeus whiteheadi, S. boops, L. australis, L. hectoris, D. meadi, D. hudsoni, S. australis and *S. elegans* appeared in the diet mixture of *Merluccius capensis*. The possible dietary contributions of these prey species overlapped (Fig. 22). However *E.*

whiteheadi had the highest mean proportional contribution (18%) to the diet of M. *capensis* (Fig. 22). *Symbolophorus boops*, *S. australis* and *S. elegans* contributed equally to the diet of *M. capensis* (14%). *Lampanyctodes hectoris* had the lowest mean proportional contribution (9%) to the diet of this predator (Fig. 22). The rest of the sources contributed similarly, ranging from 10% to 14%, to the diet of this predator (Fig. 22).

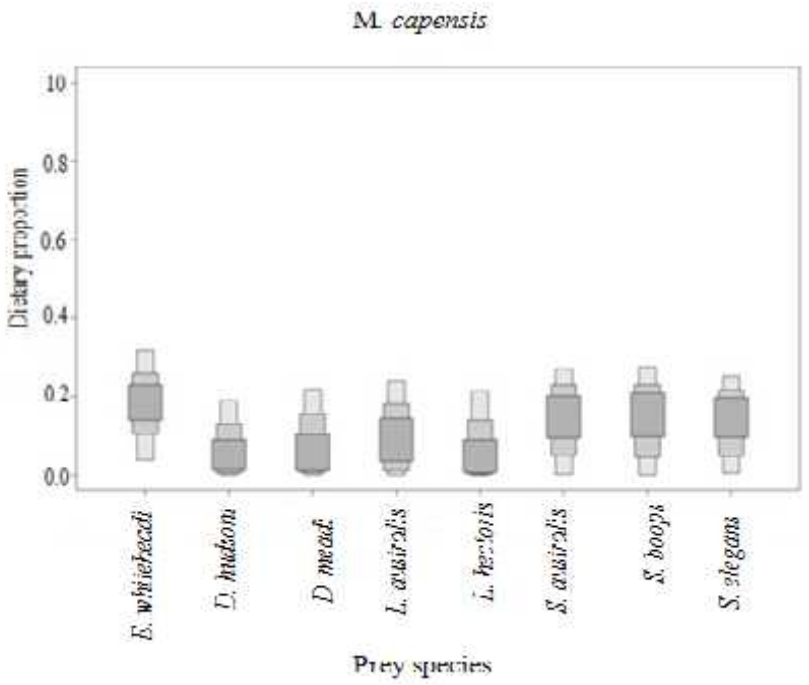

Figure 22: Contributions of *Etrumeus whiteheadi, Symbolophorus boops, Lampanyctus australis, Lampanyctodes hectoris, Diaphus meadi*, *Diaphus hudsoni*, *Sepia australis* and *Sepia elegans* to the diet of *Merluccius capensis*, as determined by a SIAR mixing model. Each box and whisker shows 95%, 75% and 50% credibility intervals.

4.6.2 Dietary contributions of different prey species to the diets of *M. paradoxus*

Diaphus hudsoni had the highest mean proportional contribution (19%) to the diet of M. *paradoxus,* indicating that it was the most prefered component in its diet, followed by both *S. australis* and *S. boops* which made an equal mean contribution of 15 %. *Sepia elegans* had the lowest mean proportional contribution (7%) to the diet of this predator (Fig. 23). The rest of the sources made similar contributions, ranging from 10% to 15%, to the diet of this predator (Fig. 23).

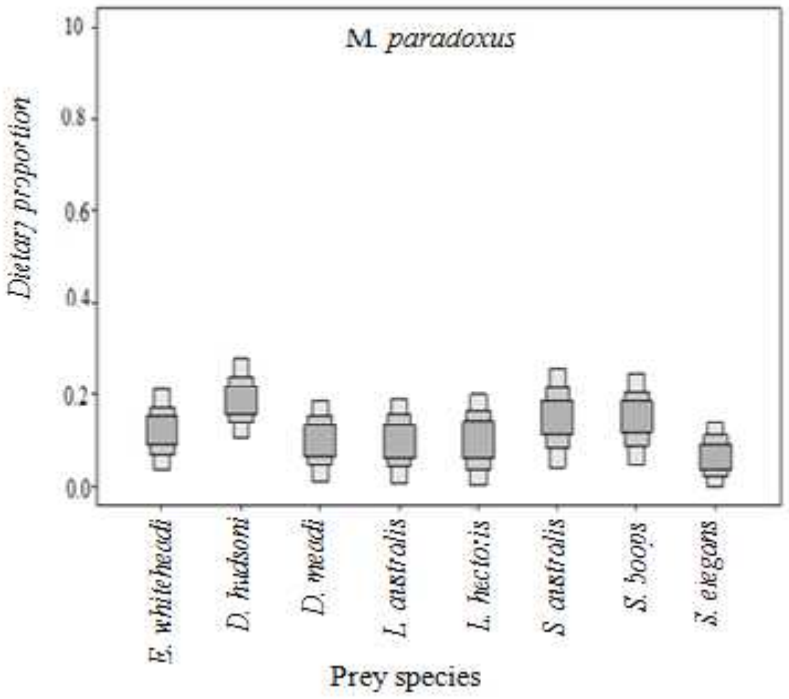

Figure 23: Contributions of the *Etrumeus whiteheadi, Symbolophorus boops, Lampanyctus australis, Lampanyctodes hectoris, Diaphus meadi, Diaphus hudsoni, Sepia australis* and *Sepia elegans* to the diet of *Merluccius paradoxus,* as determined by a SIAR mixing model. Each box and whisker shows 95%, 75% and 50% credibility intervals.

4.6.3 Dietary contributions of different prey species to the diets of *T. capensis*

Among the potential prey species analysed in this study, *E. whiteheadi* had the highest mean proportional contribution (23%) to the diet of *T. capensis*, indicating that it was the most important prey in the diet while *L. hectoris* had the lowest mean proportional contribution (6%) to the diet of this predator (Fig. 24). Other species (sources) contributed almost similarly to *T. capensis'* diet, with proportions ranging from 8% to 18% (Fig. 24).

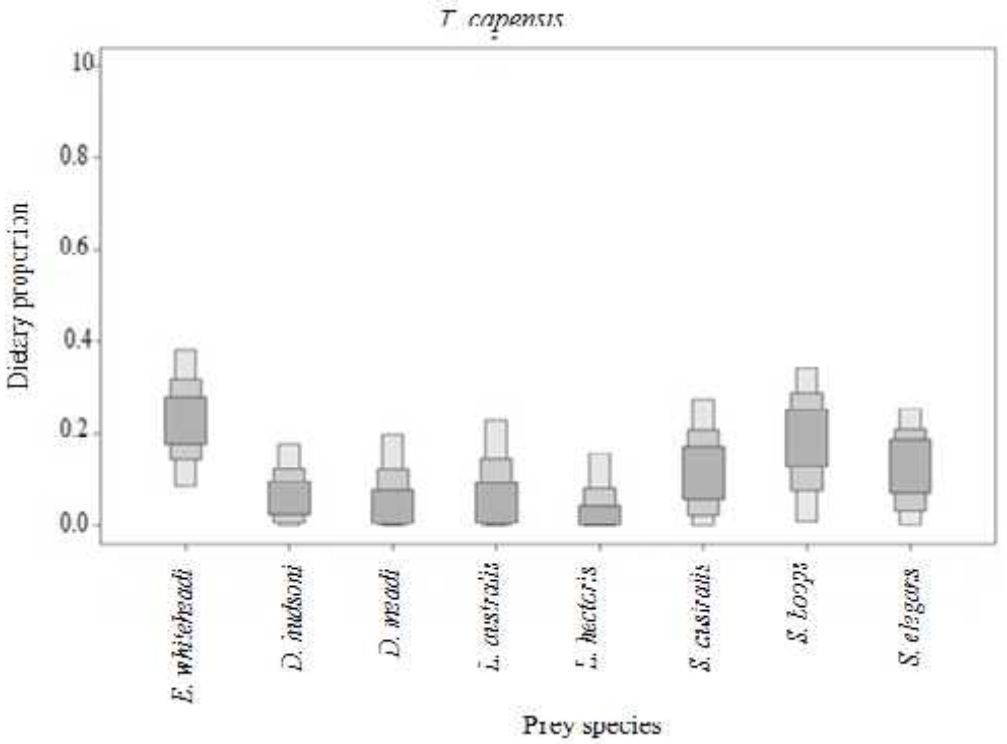

Figure 24: Contributions of *Etrumeus whiteheadi*, *Symbolophorus boops*, *Lampanyctus australis*, *Lampanyctodes hectoris*, *Diaphus meadi*, *Diaphus hudsoni*, *Sepia australis* and *Sepia elegans* to the diet of *Trachurus capensis* as determined by a SIAR mixing model. Each box and whisker shows 95%, 75% and 50% credibility intervals.

4.6.4 Dietary contributions of different prey species to the diet of *L. vomerinus*

Bayesian isotope mixing model, SIAR analysed the possible contributions of different prey to the diet of *L. vomerinus*, and showed that *E. whiteheadi* had the highest mean proportional contribution (24%) to the diet of *L. vomerinus*, indicating that it was most important components in the diet. *Diaphus hudsoni* had the lowest mean proportional contribution (4%) to the diet of this predator. Other species (sources) contributed, almost similarly to *L. vomerinus'* diet, with proportions ranging from 7% to 18% (Fig. 25).

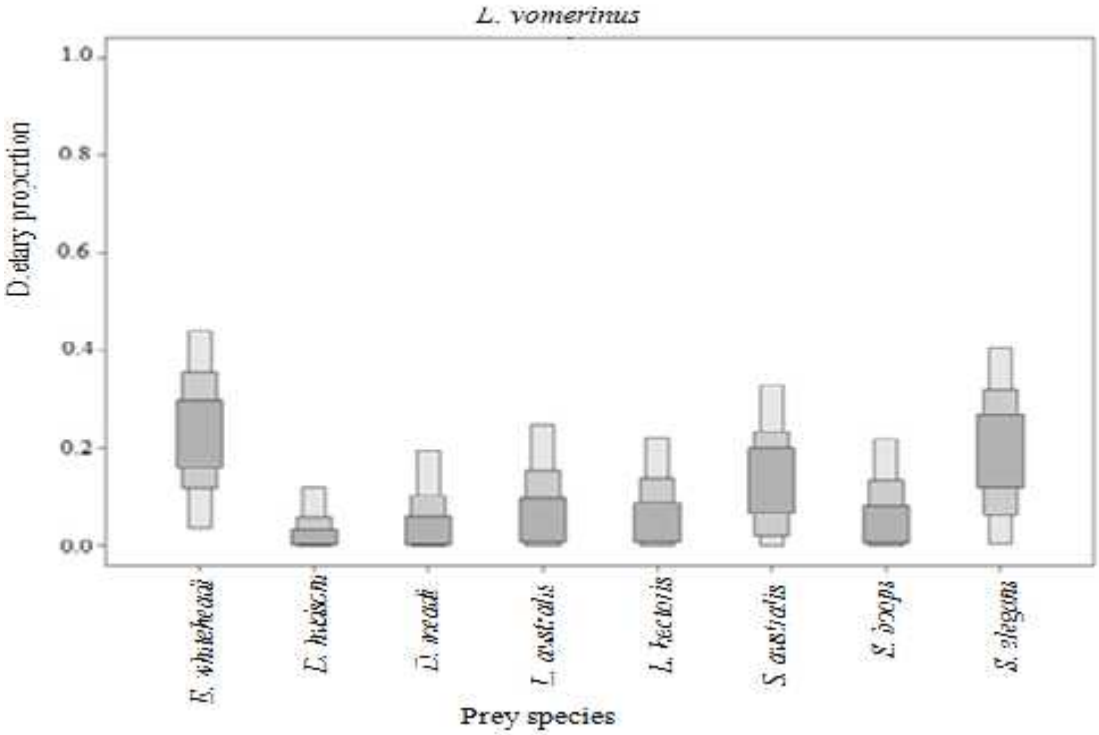

Figure 25: Contributions of *Etrumeus whiteheadi*, *Symbolophorus boops, Lampanyctus australis, Lampanyctodes hectoris*, *Diaphus meadi*, *Diaphus hudsoni*, *Sepia australis* and *Sepia elegans* to the diet of *Lophius vomerinus* as determined by a SIAR mixing model. Each box and whisker shows 95%, 75% and 50% credibility interval.

Chapter 5

Discussion

This study used stable isotopes to investigate the diets of four major commercial fish species that are also important preadators, and the trophic levels that the prey of these predators feed on in order to improve understanding of the trophic relationships that are underlying these important commercial fisheries off the Namibian coast.

5.1 Species isotopic characteristics

$\delta^{15}N$ values indicated diets of organisms and therefore reflect similarities or differences in the trophic level at which they are feeding. $\delta^{15}N$ values of the eight species are almost identical (ranging between 10.94‰ to 12.61‰, which is indicative of feeding activities directed at similar trophic levels (zooplankton). This in turn demonstrates strong dietary overlap. Euphausiids, which is a zooplankton was reported as a prey items for *S. australis* (Gibbons, 1999), *L. hectoris* (Staby & Krakstad, 2008), *E. whiteheadi* (Roel & Armstrong 1991) and other myctophids (Staby & Krakstad, 2008).

Among the myctophids, $\delta^{15}N$ was higher in muscle tissue of *L. hectoris*, probably indicating some predation on fish (Gibbons, 1999; Staby & Krakstad, 2008). Despite the size of *L. hectoris*, the possibility of it to feed on fish is not questionable because *E. whiteheadi* also was reported to only feed on zooplankton but recently few *E. encrasicolus* (fish) were found in its stomach (researher's observation). This is a slightly higher trophic level in addition to zooplankton they feed on. The higher the $\delta^{15}N$ of an

organism, the higher the trophic level it feeds on. This is due to the concentration of nitrogen in an organism that tends to build up as one moves up to higher trophic levels because the heavier isotope is preserved as tissues are being synthesized (Fry, 2006). In the nothern Benguela ecosystem, Staby and Krakstad (2008) reported the diet of *L. hectoris* as dominated by copepods, amphipods and euphausiids.

All eight species showed similar δ13C' values, which show almost similar carbon sources in their diets. Carbon ratios in tissue of different organisms differs primarily due to their carbon source that traces back to physiological processes that modify ^{13}C and the sources of CO_2 that is used in the photosynthesis process (Michener & Lajtha, 2007; Kurle *et al.,* 2011). However, all species showed much depleted δ13C' values that ranged from -17.72‰ to -16.86‰ (Table 2) compared to those of primary consumers. This is an indication of dependence on benthic prey (Iitembu, 2014).

Overall, these species, with an exception of *E. whiteheadi* had almost similar δ13C' (-17.72‰ to -17.16‰) indicating similar carbon sources. This could also possibly mean that most species were feeding more offshore, explaining why they had lower δ13C' values than those feeding more nearshore (Kurle, *et al.* 2011). Staby and Krakstad (2008) reported that *L. hectoris* feeds mostly inshore. Differences in both $\delta^{15}N$ and δ13C' may result between benthic-pelagic productions (Takai *et al.,* 2002) as well as between coastal and offshore waters (Miller *et al.,* 2008). In this study, *D. hudsoni* (-17.72‰) and *S. boops* (-17.36‰) had the most depleted δ13C' value (Table 2) indicating off-shore feeding activities. These results are in agreement with the ones reported in Staby and Krakstad (2008).

Etrumeus whiteheadi had the least depleted δ13C' value possibly indicating feeding inshore (δ13C'= -16.86‰). The $\delta^{15}N$ and δ13C' values of *E. whiteheadi* complement the results obtained by Iitembu, 2014, because they are almost identical. For instance, Iitembu, 2014 reported *E. whiteheadi* as having δ13C' value of -16.82‰. The fact that *E. whiteheadi* is a pelagic species might explain the difference in its carbon source because carbon in a diet of a consumer can come from a number of dietary carbon sources (Focken & Becker 1998).

C: N ratios of the tissue of these eight prey species are insignificantly different, possibly implying that their carbon and nitrogen assimilation efficiencies do not differ too. The species with higher C: N ratios also have higher lipid content. Initially, the C: N ratios of all eight species for this study were greater than 3.5. Usually, when C: N ratios exceed 3.5, it implies that the carbon values are affected by the presence of lipids because of individual differences in δ13C values that arise due to variation in lipid content (McConnaughey and McRoy 1979; Moody, 2007; Iitembu, 2014). The lipid affect on carbon values can be addressed by either direct physical lipid extraction (McConnaughey & McRoy, 1979) or through a mathematical normalization equation (Post *et al.,* 2007) to standardize the content of lipid and hence correct the carbon values that are assumed to be bias (Post *et al.,* 2007). For this study, physical lipid extraction was not done because, even though it reduces the influence of lipids on δ13C values, it also has a negative effect during stable isotope analyisis, since it causes fractionation in $\delta^{15}N$ (Post *et al.,* 2007), however, the values were mathematically corrected for lipid and new lipid-corrected δ13C' values were used.

5.2 Trophic levels of prey species

One of the main objectives of this study was to determine the trophic levels of the eight prey species and to compare them. During calculations of trophic levels, the trophic enrichment factor was assumed to be a 3.4‰ increase in $\delta^{15}N$ per trophic level (Minagawa & Wada 1984; Post 2002b). $\delta^{15}N$ of mytilid bivalves feeding at a trophic level of 2.0 were taken as $\delta^{15}N$ base in this study because other than the bivalves being primary consumers they also have prolonged isotopic turnover rates that can integrate seasonal changes (Fukumori *et al.,* 2008). Using primary consumers as baseline indicators is particularly important in order to minimize error in estimates of the trophic position of higher level species (Vander Zanden & Rasmussen, 2001).

Results from this study indicated that seven of the eight species analysed appear to feed at low trophic levels (between 2.51 – 2.75) (Table 3), indicating that they are all secondary consumers. These results correspond to Connan *et al.*, (2010), who reported that most myctophid*s* feed on zooplankton, making them secondary consumers. However, *L. hectoris* of the Myctophidae family fed at the highest relative trophic level compared to the other species analyzed (mean $\delta^{15}N$ of 12.61 ‰, trophic level 3.00) (Table 3). This might imply that, although *L. hectoris* is also a secondary consumer, it fed on other secondary consumers as well. In this study, *E. whiteheadi* fed at a trophic level of 2.67, which is similar and comparable to a trophic level of 2.83 calculated in Iitembu (2014), in the same location, using the same analysis method. However, *E. whiteheadi* was reported to feed at a level of 3.64 (Van Der Lingen & Miller, 2011) for the southern Benguela ecosystem. This contrast results from the current study, indicating

that the northern and southern Benguela ecosystems are different in terms of its food web structure and energy flow.

Diaphus meadi, S. australis and *S. elegans* all fed at exactly the same level (TL = 2.75). Since organisms at the same trophic level have fairly similar trophic fractionation, these species have similar and comparable diets. All myctophids in this study feed at trophic levels between 2.51 and 2.75, corresponding with documented findings (Watanabe, Moku, Kawaguchi, Ishimaru, & Ohno, 1999; Wang & Chen, 2001). The above also support finding from different studies that indicated that myctophids feed on zooplankton such as copepods, amphipods, euphausiids and fish (Gibbons, 1999; Staby & Krakstad, 2008).

This study calculated 2.51 as the trophic level for *S. boops* and this does not support the results of Hulley (1990) who found that *S. boops* feed at a trophic level of 3.45. Again these two studies were conducted at different times, in 1990 and 2014, which might explain the differences in trophic level because a trophic level of an organism is not fixed in time. Furthermore, samples used in this study were obtained from different stations/locations compared to those used in Hulley (1990) and these different locations might have different prey composition and prey availability, possibly explaining different trophic levels calculated for the same species.

The trophic levels presented in this study are also slightly different from those of other studies (Van Der Lingen & Miller, 2011; Iitembu *et al.,* 2012; Iitembu, 2014). These differences are not surprising because these species are from different areas, they were not collected during the same period and the methods used (isotope analysis vs. stomach

content analysis) are different. For future studies trophic level calculations should be done separately for different length classes because trophic levels change with age of species for the reason that fish muscle has an isotopic turnover that mirrors growth and metabolic tissue replacement and therefore fluctuates depending upon the type of species and age of the fish (Perga & Gerdeaux, 2005; Buchheister & Latour 2010; Weidel, Carpenter, Kitchell & Vander Zanden, 2011; Iitembu, 2014). Weidel *et al*. (2011) also reported that juvenile fish grow faster and thus tend to have faster isotope turnover times than do adult fish that grow slowly and whose isotopic turnover is due mostly to metabolic tissue replacement.

5.3 Resolving relationships among the species

The δ^{15}N and δ13C' values of the eight prey species in the northern Benguela overlapped as initially hypothesized. Significant differences were found in δ13C' values of the different prey species. Differences in δ^{13}C values may result from the primary carbon source of food webs in which these prey species are found. Some of the analysed species, for example *D. meadi, S. boops* and *D. hudsoni* are off-shore species (Staby and Krakstad 2008), while other species such as *L. hectoris* feeds inshore. The prey items found offshore are not necessarirly similar to the ones found off-shore thus these differences reflected in these prey species. There are differences in δ^{13}C values of marine environments, for instance, δ^{13}C values are reported to increase as latitude decrease in the northern hemisphere (Kurle *et al.*, 2011). Kurle *et al.*, (2011) reported that organisms that feed close to the shore tend to have higher δ^{13}C values compared to

those that feed offshore. Furthermore, the geographic positions and depth strata where these eight sprey species were found are different (Fig. 13), thereby explaining differences in δ13C' values. Whether a food web is fueled by benthic or pelagic production, it influences the $\delta^{13}C$ values (Miller *et al.*, 2008), which might have been the case in this study, seeing that some species are pelagic such as *E. whiteheadi* and others are benthic such as *D. hudsoni*. The significant differences found in terms of $\delta^{15}N$ values of the different prey species, were few. This implies that the species which were significantly different from each other fed at different trophic level. Significant difference in terms of $\delta^{15}N$ between *L. hectoris* and other species as well as between *S. australis* and others species clearly indicate that *L. hectoris* and *S. australis* were significantly different from the other species. The rest of the species showed no significant differences between each other in terms of $\delta^{15}N$, indicating that they feed at more or less the same trophic level. The Benguela current is known for its strong upwelling, which brings nutrient rich waters to the surface and in the process mixing cross-shelf and offshore waters. This mixing of waters leads to homogeneous mixture of food sources for different organisms (Iitembu, 2014), thereby resulting in similar $\delta^{15}N$ signatures between organisms. Furthermore, the trophic levels among the rest of the species changes little and for three species (*D. meadi, S. australis* and *S. elegans*) the trophic level is exactly the same. These results are also in agreement with previous studies (Staby & Krakstad, 2008) that indicated that these species are at the same trophic level except for *L. hectoris*.

5.4 Trophic niche of prey species

One of the main aims of this study was to determine the trophic niche of the eight prey species analysed. In order to do this it was important to study the δ ^{15}N range, δ13C' range and Total area (TA) of these species (*E. whiteheadi, S. boops, L. australis, L. hectoris, D. meadi, D. hudsoni, S. australis* and *S. elegans*), because these are community-wide metrics that show specific aspects of trophic structure within a food web (Layman *et al.,* 2007).

The results showed the convex hull area is a representation of the total extent of trophic diversity within a food web (Layman *et al.*, 2007). *Lampanyctodes hectoris* had the most enriched δ^{15}N, while *S. boops* had the most depleted δ^{15}N. *Symbolophorus boops* had a larger range in δ^{15}N among all other prey species, suggesting more trophic levels and thus a greater degree of trophic diversity. This is possible because *S. boops* is a bathypelagic species (Bianchi *et al*., 1999). The closer one moves to the shore areas, the higher the species diversity (Iitembu, 2014). This might mean that *S. boops* has more diverse carbon sources then the rest of the species analysed. There is very little information on *S. boops* (authers's observation). *Diaphus hudsoni* had the lowest δ^{15}N (0.76) indicating a lower trophic diversity in its diet (Layman *et al.,* 2007). *Etrumeus whiteheadi* had the highest δ13C' range of 8.64, suggesting multiple basal resources in its food web feed. Van Der Lingen and Miller (2011) suggested that *E. whiteheadi* feed on large zooplankton. *Diaphus hudsoni* had the lowest range of δ13C' (1.74) possibly pointing at a similar niche at the base of a food web (Layman *et al.,* 2007). *Diaphus*

hudsoni is reported to feed on hyperbenthic and pelagic crustaceans (Lipin´ ski *et al.*, 1991), thereby explaining this low carbon range (1.74).

Generally a larger range in $\delta^{15}N$ among consumers suggests more trophic levels and thus a greater degree of trophic diversity perhaps implying that they feed on prey from various trophic levels. Thus, the range of δ13C' and $\delta^{15}N$ indicated that *E. whiteheadi* had a higher range of carbon sources as well as enhanced trophic diversity in its feeding patterns compared to the other prey species. This information is not found in available litreture on *E. whiteheadi*, which is not suprising because *E. whiteheadi* is not a commercially important species and therefore few studies have been carried out on this species.

Total area (TA) represents a measure of the total amount of niche space occupied, and is thus a proxy for the total extent of trophic diversity within a food web. TA is influenced by species with extreme positions on either the $\delta^{13}C$ or $\delta^{15}N$ axis (or both), and thus typically will be correlated to some degree with these two metrics.

The comparison of trophic niche sizes of the eight prey species (Fig. 16) indicated that *E. whiteheadi*, *S. boops*, *D. meadis,* and *L. hectoris* occupied a relatively large area of isotopic niche space relative to the remaining four prey species. *Etrumeus whiteheadi* had the second widest trophic niche, the widest being that of *S. boops* (Fig. 16). These results corresponds with that of Mqoqi, Lipin´ski and Salvanes (2007), who found *S. australis* to be an opportunistic feeder, with a diet that is highly dependent on whatever prey is abundant, and is as a result influenced by the environment. A broader isotopic niche in these prey species may depict feeding generalization that hint at a high degree

of feeding competition with other species. Another possible explanation for the trophic niche overlap among these prey species could be that they all share euphausiids and mysids as their prey although the predation intensity on these food items differs from species to species. Mqoqi et al. (2007) reported that euphausiids and mysids are the main prey items for *S. australis*. In contrast, *S. elegans*, *S. australis* and *D. hudsoni* occupy a narrower isotopic niche which can also indicate feeding specialization that can reduce competition with other species. Niche overlap may cause the competitive exclusion of a species, or species may avoid exclusion by employing isolation mechanisms (Diamond, 1978), whereby such a species distance itself from others. The main aim for isolation mechanisms is to reduce overlap of resources that are shared (Diamond, 1978). Therefore, the degree of species overlap in the utilization of resources such as food, space, and shelter has become a valuable approach in studying not only community structure but species co-existence as well.

5.5 Trophic niche and trophic level of predator species

The trophic niches for *M. capensis*, *M. paradoxus* and *T. capensis* overlap (Fig 21), indicating that these consumers all compete for the same resources. This corresponds to the habitat overlap of these three species (Bianchi *et al*., 1999) which explains why they feed on the same prey items. The trophic niche of *M. paradoxus* is however, the smallest while that of *T. capensis* is the widest (Fig. 20 and 21). *Lophius vomerinus'*s trophic niche does not overlap with that of the other three predator species, probably indicating another source of food not analysed in this study.

Merluccius paradoxus and *M. capensis* occupied a relatively small area of isotopic niche space relative to *L. vomerinus* and *T. capensis* (Fig. 21). These narrower isotopic niches in both hake species may point out feeding specialization that can reduce competition not only with *L. vomerinus* and *T. capensis* (Iitembu, 2014), but also with other predators that share the same food resources. However, the individual variability in isotopic values shows high degrees of overlap among these predators.

The $\delta^{15}N$ values of the predators (*M. capensis*, *M. paradoxus*, *L. vomerinus* and *T. capensis*) in table 8 are lower than those of the prey species in table 2, except for *L. vomerinus*. This might give an impression that the predators fed at lower trophic levels than their prey species, however, it should be noted that the $\delta^{15}N$ values are averages. Some organisms, fish included, change their diet (ontogenetic shifts) as they develop from juveniles to adults. Iitembu (2014) noted ontogenetic shifts in feeding habits of both hake species (*M. paradoxus* and *M. capensis* and *T. capensis*. If the $\delta^{15}N$ values of predators were presented per length class, a difference in $\delta^{15}N$ values would have probably been noted, however, the objective of this study was not to categorize the predators into length class but to assess as a predator.

Results indicated that *L. vomerinus* fed at the highest trophic level (Table 8) probably confiming its piscivorous behaviour, targeting demersal fish as reported in Walmsley and Sauer (2005). *Merluccius capensis* was reported to feed at a trophic level of 4.7, in Schukat *et al.,* (2013), while in this study it fed at 2.15. This deviation might be due to temporal difference since Schukat *et al.,* (2013) collected the samples in 2009, while samples for this study were collected in 2013. Differences might have arised also from

the methods used. Schukat *et al*., (2013) corrected the stable isotope analysis for ^{13}C by performing lipid correction in their samples, while samples in this study were not corrected for lipids. Additionally, this study indicated that *T. capensis* fed at a 2.52 trophic level, this implies that it is a secondary consumer. These results correspond to the results of Barange *et al.* (2005) who reported that *T. capensis* feeds mainly on copepods and euphausiids, which are zooplankton.

5.6 Dietary contributions of different prey species to the diets of commercial species (using mixing models)

Studies on stomach content analysis indicated that *E. whiteheadi, S. boops, L. australis, L. hectoris, D.meadi, D. hudsoni, S. australis* and *S. elegans*are are common prey species for *M. capensis, M. paradoxus, L. vomerinus* and *T. capensis* (Macpherson & Roel, 1987; Traut, 1996 Walmsley *et al,* 2005). The feasible contribution of the eight prey species to the diet of the four predators was analysed. Enhanced understanding of the feeding patterns including diet composition, sources of variability, and trophic interactions of the major fish predators is crucial for determining their ecological roles in a given marine ecosystem (Mohanraj & Prabhu, 2012). Mixing models such as Multiple-isotope mixing models have been employed to study the relative contribution of the sources for predator's who have two or more sources of energy for growth (Jardine *et al.*, 2003).

Results from mixing models reduce biases associated with stomach content analyses because of the use of time and space-integrated isotopic data (Layman & Allgeier,

2012). Because *M. capensis, M. paradoxus, L. vomerinus* and *T. capensis* are generalist feeders and therefore are a mixture of diverse prey from nearshore-offshore and benthic-pelagic habitats (Iitembu, 2014), it is vital to resolve and quantify the possible contributions of each prey species to the isotopic mixture. *Etrumeus whiteheadi, S. boops, L. australis, L. hectoris, D. meadi, D. hudsoni, S. australis* and *S. elegans* all contributed to the diets of *M. paradoxus, M. capensis, L. vomerinus* and *T. capensis.* Results from this study indicated that the dietary contributions of these species overlapped. Possible contributions of different prey to the isotopic mixtures of the three commercial species showed dietary overlap among all three species. *Etrumeus whiteheadi* was dominant in the diet of all *M. capensis, L. vomerinus* and *T. capensis* (18, 24% and 23%, respectively) indicating that it probably offers the best nutritional input for these predators and is therefore favoured (Mohanraj & Prabhu, 2012). The possible contribution of each species is important because data on diet composition are functional in the creation of trophic models as a tool to understand complex coastal ecosystems (Mohanraj & Prabhu, 2012), and this would put resource managers in a better position to sustainably manage commercial species.

Information of feeding ecology of hake such as feeding time, prey preferences, exist in literature on stomach contents analysis; this information is vague especially as far as identifying the relative contributions of different prey is concerned. This shortcoming is due to the opportunistic feeding behaviours of both *M. paradoxus* and *M. capensis* (Punt *et al.,* 1992), and the natural variability associated with prey availability and densities (Macpherson & Gordoa, 1994). One of the core objectives of this study was to elaborate

on the possible contribution of some of the selected prey species to the diet of both *M. paradoxus* and *M. capensis* through the use of stable isotope mixing models. Both *M. paradoxus* and *M. capensis* fed on all eight prey species (*E. whiteheadi, S. boops, L. australis, L. hectoris, D. meadi, D. hudsoni, S. australis* and *S. elegans*). *Diaphus hudsoni* was the most important component in the diet of *M. paradoxus* accounting for mean proportional contribution of 19%, while *E. whiteheadi* was a dominant contributor, with the highest proportional contribution of 18% to the diet of *M. capensis* with credible intervals of 0.04-0.31%. The observed dominace of *E. whiteheadi* in the diet of the three predators in this study is not suprising because *E. whiteheadi* is increasingly becoming an important prey item for many predators such as seals and sea birds in Namibian waters. This is observation is based on scats analysis (feacal analysis). Although Pillar and Barange (1997) reported that *M. paradoxus* move from the bottom to feed on pelagic prey, results from this current study do not confirm this, because the diet of *M. paradoxus* in this study is dominated by *D. hudsoni* which is not a pelagic species. Pillar and Barange (1997) also reported that smaller *M. capensis* consumed pelagic fish, mainly round herring (*E. whiteheadi*). This is confirmed in this current study, since it is also the dominant prey species for *M. capensis.* It should be noted that although *E. whiteheadi* had the highest mean proportional contribution (18%) in the diet of *M. capensis* (Fig. 22) it does not imply that it is the most important prey in that predator's diet. This can imply that, among the prey species analysed, it was the most consumed but there are other preys also (such as anchovy (*E. encrasicolus*) and squid)

that were not analysed in this study. For instance, Barange *et al.* (2005) reported that *T. capensis* is an important food item for hake species.

Analysis of the diets of these two predators showed that both *M. paradoxus* and *M. capensis* are generalists and therefore consume a mixture of various prey from nearshore or offshore and benthic or pelagic habitats and this is in agreement with the results reported by Iitembu *et al*. (2012). Roel and Macpherson (1988) stated that the diet of hake is determined by the availability of prey and therefore it changes, given different environmental conditions and such changes can be seasonal. Food preference of predatory fish is complex and is influenced by many factors such as prey accessibility and mobility, prey abundance, prey energy content, prey size selection and seasonal changes (Mohanraj & Prabhu, 2012), thus different results migtht be obtained if studies of this kind are carried out seasonally.

Etrumeus whiteheadi had the highest mean proportional contribution (23%) to the diet of *T. capensis,* indicating that it was the most important component in the diet. Both *T. capensis* and *E. whiteheadi* are mid-pelagic species and this might increase the chances of *T. capensis* feeding more on *E. whiteheadi* than on other prey species.

Results from this study indicated that although each prey species analysed notably contributed to the diet of *T. capensis*, it was in small amounts, which was similar to what Andronov (1983) reported. He reported that *T. capensis* feed to a lesser extent on lantern fish. *L. hectoris* had the lowest mean proportional contribution (6%) to the diet of this predator. Other species contributed almost similarly to *T. capensis'* diet, with proportions ranging from 8% to 18% (Fig. 24). It is clear that none of the analysed prey

species dominated the diet of *T. capensis.* Heymans *et al.* (2004) reported that its diet is predominantly euphausiids and the species is mainly feeding at higher trophic levels (Heymans *et al.*, 2004), 95% of euphausiids is reported to dominate the diet of adult *T. capensis* off Namibia (Konchina, 1986, Boyer & Hampton 2001). This may explain why each of the species analysed contributed very little to the diet mixture of *T. capensis.* The results for analysis of the diet of *T. capensis* revealed that its diet was more varied compared to that of *M. capensis* and *M. paradoxus*. The trophic level of *T. capensis* in the northern Benguela ecosystem for this study was reported to be at 2.52 (Table 8), which is low compared to that of the southern Benguela ecosystem which is reported to be around 3.6 for juvenile *T. capensis* and 3.7 for adult *T. capensis* based on Mass-balanced models constructed using the Ecopath software (Shannon *et al.*, 2003). Moloney *et al.* (2005) reported that the northern Benguela ecosystem is different and its species are at different trophic levels compared to other ecosystem.

Lophius vomerinus fed on all eight prey species. Possible contributions of all prey species analysed were minimal (below 25%). This could be explained by the fact that *L. vomerinus* is known to feed on various bottom-living fishes (Bianchi *et al.*, 1999); however, none of the analysed species are bottom-living fishes. *Lophius vomerinus* is a piscivorous fish that ambushes its prey (Walmsley *et al.*, 2005). The eight prey species are all active benthic feeders that are not likely to be lured by *L. vomerinus* and this might explain why each of these prey species contributed very little to the diet of *L. vomerinus.* However, *E. whiteheadi*, had the highest mean proportional contribution (24%) to the diet of *L. vomerinus* (Fig. 25), indicating that it was the most important

component in its diet compared to the rest of the species analysed. However, *E. whiteheadi* is a pelagic species and live far from the bottom, where *L. vomerinus* is found. It is more probable that the high proportion of *E. whiteheadi* in the diet of *L. vomerinus* proposed by this mixing model may be an over estimation. Another possible explanation is that *E. whiteheadi* might have been consumed by other preys of *L. vomerinus* such as hake and thus it ended up in the diet of *L. vomerinus.* However, other authors (Walmsley *et al.,* 2005; Dooley *et al.,* 2010) reported that *L. vomerinus* feeds on small fish, including *M. capensis* and *E. whiteheadi* and this corresponds with this study's findings.

Walmsley *et al.* (2005), reported that both *M. paradoxus* and *M. capensis* dominate the diet of *L. vomerinus* possibly explaining why the analysed prey species contributed very little (ranging from 4% to 24%) to the diet of *L. vomerinus*. In contrast, *D. hudsoni* had the lowest mean proportional contribution (4%) to the diet of this predator, indicating that it is the least of *L. vomerinus'* preferred prey item. Other species contributed almost similarly to *L. vomerinus'* diet, with proportions ranging from 4% to 20% (Fig. 25). The absence of a dominant prey species in the diet of *L. vomerinus* in this study is consistent with the findings of other researchers such as Walmsley *et al.* (2005) and Fariña *et al* (2008) who reported this fish to be an opportunistic and non selective feeder with a common feeding strategy.

5.7 Limitations to the study

Like any other research, this study too had limitations. One notable limitation was the number of surveys conducted, since they were only carried out once per year for *L. vomerinus* and twice per year for *M. paradoxus*, *M. capensis* and horse mackerel. Sampling was done more during the day than at night; this might have affected the quantities of the samples caught given that fish are mostly active at night as attested in literature review. Another limiting factor for this study was that, during the surveys the types of fishing gear used were specific for target species, so mesh sizes were big allowing these small fishes to escape. field sampling; which was restricted to the pre-determined stations and route of the sampling vessel; hence the sampler had little control, the depth and positions were predetermined, more samples could have been obtained and a variety of samples could have been sampled had the sampler sampled all possible depths and locations.

Chapter 6

Conclusions and Recommendations

This study demonstrated the usefulness of stable isotope analysis in elucidating trophic aspects and giving insight into trophic levels in food webs, calculation of trophic niches, assessing trophic interactions, determining the relationship between δ^{15} N and δ^{13}C and quantification of possible contributions of prey species to the diet of their predators in the northern Benguela ecosystem off Namibia.

All species had almost similar δ13C' values indicating similar carbon sources except for *E. whiteheadi* that relied on a different source of carbon, given that it is a pelagic species and found in shallower waters compared to the rest of the species studied.The C: N ratio of all eight species was greater than 3.5, indicating high levels of lipids, which might affect carbon values. However, the δ^{13}C values were mathematically corrected for variations in lipid and new lipid treated values were obtained. This emphasizes the importance of lipid correction.

All eight prey species had trophic niches of different sizes. Their niche sizes overlapped, indicating similar feeding patterns. Results on trophic niche quantification might have been affected by the differences in sample sizes of species analysed which ranged from 7 to 117. *Lampanyctodes hectoris* had the most enriched ^{15}N values while *S. boops* had the most depleted ^{15}N values. *Symblophorus boops* is trophically more diverse in terms of its ^{15}N range. *Merluccius paradoxus* and *M. capensis* occupied a relatively small area

of isotopic niche space compared to *L. vomerinus* and *T. capensis,* possibly indicating reduction in feeding competition with *L. vomerinus* and *T. capensis*. Results from the Bayesian isotope mixing model indicated that *M. paradoxus, M. capensis* and *T. capensis* fed on all eight prey species (*E. whiteheadi, S. boops, L. australis, L. hectoris, D. meadi, D. hudsoni, S. australis* and *S. elegans*) without a particular preference of one over the others. However, this may change depending on their availability. Overall, *E. whiteheadi* dominated the diet of both *M. capensis* (18%) and *T. capensis* (23%), while *D. hudsoni* (19%) dominated the diet of *M. paradoxus.* Although results indicated no dietary preference of the four commercial species, it should be noted that the food preference of predatory fishes is complex and is influenced by many factors such as prey accessibility and mobility, prey abundance, prey energy content, prey size selection and seasonal changes, amongst others. Furthermore, the feeding habits of a juvenile animal, and consequently its trophic level, can change as it grows up. Since fish diet is highly linked to fish size which can in turn have an influence on prey preference, prey diversity, feeding behavior or feeding rate, it is strongly recommended that further work should consider these factors. Results from the current study indicated that each analysed species contributed very little to the diet of *L. vomerinus.* It therefore remains unclear as to what prey monkfish consumes in large amounts as documented by Walmsley, Leslie & Sauer (2005). Thus, future research should investigate the feeding ecology of *L. vomerinus.*

The use of stable isotopes in this study was performed with relative ease and results obtained were very useful. Accurate use of fractionation factors and turnover rates is

crucial for the findings of isotope based trophic studies to help direct future research and improve parameters such as natural mortality as one of the inputs into the stock assessment models. Future research should as well focus on determining species-specific fractionation factors and isotopic turnover rates. Through stable isotope approach, it was possible to obtain meaningful insights to address the objectives of this study, however, it was realized that trophic interactions are highly complex, non-linear and may vary with time. There is therefore a need for more long term studies to fill the gaps in the knowledge of these predators and their prey which integrate small as well as large-scale temporal and spatial sampling within the Northern Benguela ecosystem.

While the use of stable isotope analysis provided important information on trophic relationships among prey species in this study, it failed to provide a time-integrated view of prey species' diet and neglected recent feeding activities that are accessible only through the use of stomach content analyses. Ideally there should be a combination of stomach content analysis, application of fatty acids as biomarkers and stable isotope analysis to improve understanding of the feeding ecology of both predator and prey species.

This study carries limitations inherent in the use of stable isotopes in ecological studies, such as the potential errors involved in using average trophic fractionation factors of 3.4‰ for $\delta^{15}N$ and 0.39‰ for $\delta^{13}C$ (Post, 2002b). Jackson *et al.*, (2011) stressed that one of the shortcomings identified in using stable isotope analysis to determine trophic niche size, is that application of the metrics to single community group members is susceptible

to sample size, mainly across the range of sizes frequently encountered in ecological studies ($n < 50$).

This study provided evidence that *E. whiteheadi*, Myctophidae and *Sepia* spp. are on the same trophic level except for *L. hectoris* that fed at a slightly higher trophic level (3.00). *Etrumeus whiteheadi*, Myctophidae and *Sepia* spp. are secondary consumers in the marine ecosystem and possibly also feed on the tertiary consumer level. *Etrumeus whiteheadi*, Myctophidae and *Sepia* spp. are crucial in the food web, since they form a link between primary and tertiary consumers and play an important role in the energy flow within the system. If their stocks are reduced, the demersal fishing industry might collapse. *Etrumeus whiteheadi,* Myctophidae and *Sepia* spp. contribute equally to the diets of hake, monkfish and horse mackerel however; none of the prey species dominated the diets of the predator species studied. This study has the potential to assist sustainable fisheries research to establish a predator-prey relationship and can be seen as an initial attempt to improve Fisheries Management through ecosystem management approaches.

Chapter 7
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Appendix 1

The following section shows the table for number of collected samples of different species (*Etrumeus whiteheadi*, Myctophidae (*S. boops, L. australis, L. hectoris, D. meadi* and *D. hudsoni*) and *Sepia* spp. (*S. australis* and *S. elegans*) per station.

Appendix 1.1: number of collected samples of different species per station

		Sepia spp.		Myctophids				
Station number	*E. whiteheadi*	*S. elegans*	*S. australis*	*S. boops*	*D. meadi*	*L. hectoris*	*L. australis*	*D. hudsoni*
2	0	0	2	0	0	0	0	0
3	0	0	5	0	0	0	0	0
4	5	0	0	0	0	0	0	0
5	5	0	0	0	0	0	0	0
6	5	0	0	0	0	0	0	0
8	5	0	0	0	0	0	0	0
10	5	0	0	0	0	0	0	0
11	5	0	2	0	0	0	0	0
12	5	0	5	0	0	0	0	0
13	0	0	5	0	0	0	0	0
14	0	0	5	0	0	0	0	0
15	0	0	5	0	0	0	0	0
16	5	0	5	0	0	0	0	0

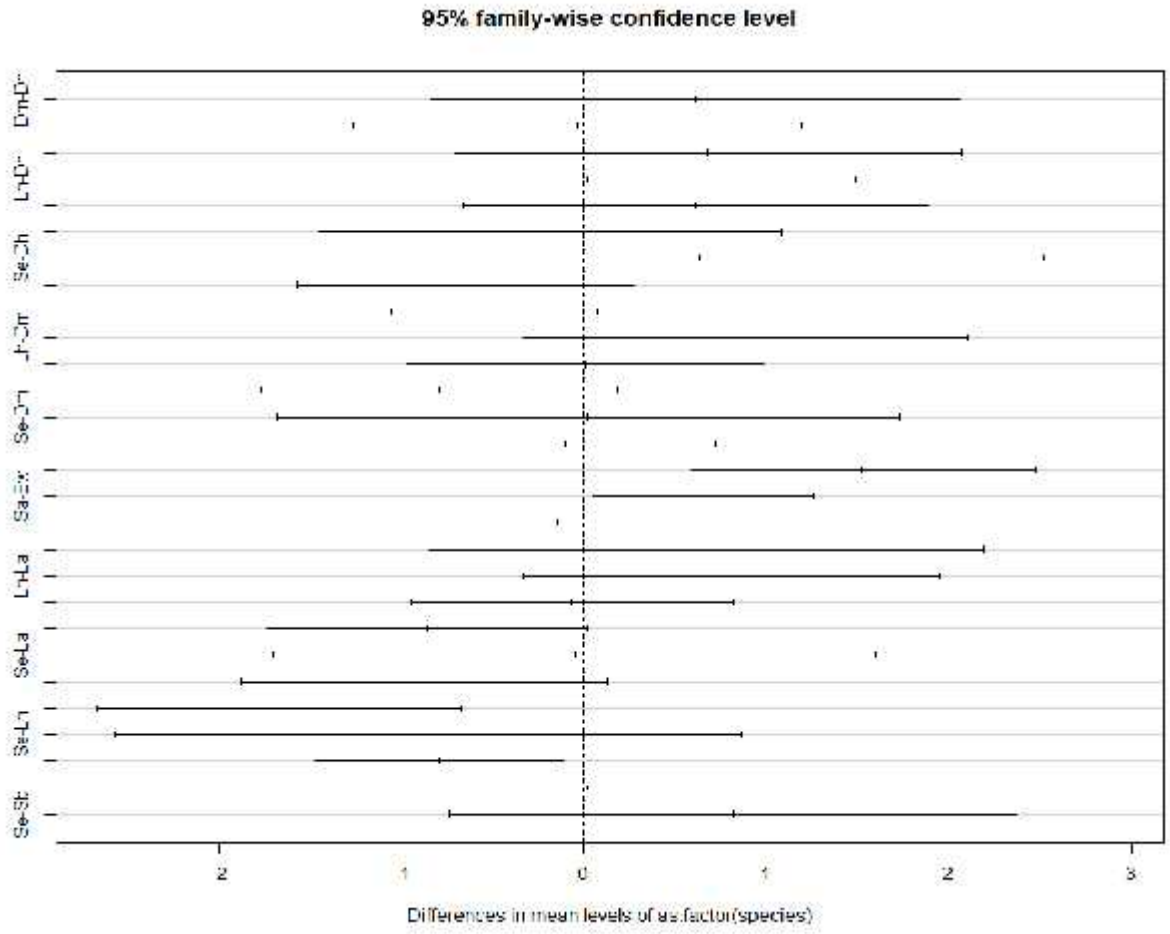
17	6	0	5	0	0	0	0	0
18	0	0	0	3	0	8	3	0
20	5	0	0	0	0	0	6	0
21	0	0	0	0	0	0	0	0
22	0	0	5	0	0	0	0	0
23	0	0	5	0	0	0	0	0
24	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0
26	0	0	0	4	0	0	0	0
27	0	0	5	8	0	0	7	0
28	0	0	5	0	0	0	0	0
31	0	0	5	0	2	0	0	0
32	0	0	0	7	0	5	0	0
38	0	0	0	0	0	5	0	0
46	0	0	0	8	0	0	0	0
48	0	0	0	0	0	2	0	0
62	0	0	0	0	0	0	5	0
65	0	0	0	0	0	0	4	0
66	0	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0
68	0	0	0	0	0	0	0	0
69	0	0	0	0	0	0	0	0

79	0	0	0	1	0	0	0	0
80	0	0	0	1	0	0	0	0
88	0	0	0	5	0	0	0	0
90	0	0	0	1	0	0	0	0
104	0	0	0	0	0	0	3	0
105	0	0	0	5	0	0	0	0
126	0	0	0	3	0	0	0	0
131	0	0	0	5	0	0	0	0
137	0	0	0	0	5	0	0	0
144	0	0	0	0	6	0	0	0
145	0	0	0	0	7	0	0	0
152	0	0	0	5	0	0	0	0
153	0	0	0	5	0	0	0	0
154	0	0	0	5	0	0	0	5
155	0	0	0	0	0	0	0	6
167	0	0	0	0	1	0	0	0

Appendix 2

The following section shows the figure for Tukey multiple comparisons of means of δ^{15}N δ13C' and among different species

Appendix 2.1:



Tukey HSD test for δ^{15}N among species [*E. whiteheadi* (Ew), Myctophidae (*S. boops* (Sb), *L. australis* (La), *L. hectoris* (Lh), *D. meadi* (Dm) *and D. hudsoni* (Dh) and *Sepia* spp. (*S. australis* (Sa) *and S. elegans* (Se))]. Horizontal lines represent data range.

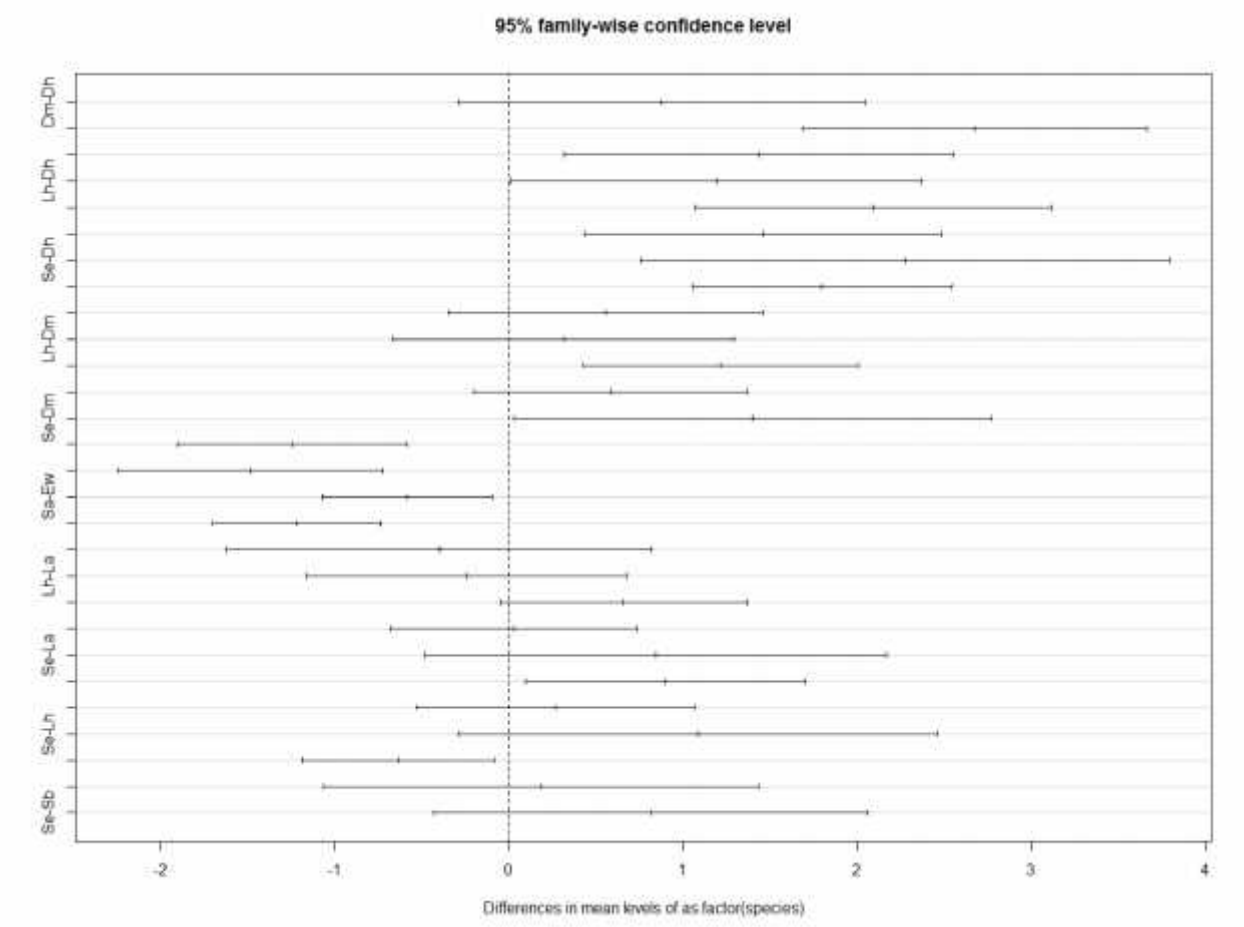
Appendix 2.2: Tukey multiple comparisons of means of $\delta^{15}N$ among *E. whiteheadi* (*Ew*), *S. boops* (*Sb*), *L. australis*, *L. hectoris*, *D. meadi*, *D. hudsoni*, *S. australis* and *S. elegans*.

Species	Diff	lwr	Upr	p adj
Dm –Dh	0.6083831	-0.8450844	2.06185062	0.9068409
Ew-Dh	0.0385497	-1.2700997	1.19300024	1
La-Dh	0.6784974	-0.7111137	2.06810849	0.8126985
Lh-Dh	1.4863695	0.02046452	2.95227457	0.0442131
Sa-Dh	0.6105374	-0.6640824	1.88515707	0.8273315
Sb-Dh	0.1865197	-1.4583038	1.08526437	0.9998364
Se-Dh	0.6280974	-1.2600123	2.51620707	0.9720112
Ew-Dm	0.6469328	-1.5724262	0.27856048	0.3962568
La-Dm	0.0701143	-1.0572018	1.19743037	0.9999995
Lh-Dm	0.8779864	-0.3421366	2.09810942	0.357308
Sa-Dm	0.0021542	-0.9799242	0.98423263	1
Sb-Dm	0.7949028	-1.7732981	0.18349244	0.2083561
Se-Dm	0.0197143	-1.6846274	1.72405601	1
La-Ew	0.7170471	-0.1045301	1.53862434	0.1380656
Lh-Ew	1.5249193	0.58001311	2.46982544	0.0000369
Sa-Ew	0.6490871	0.0419417	1.25623247	0.0266344
Sb-Ew	-0.14797	-0.7491395	0.45319958	0.9952852
Se-Ew	0.6666471	-0.8528687	2.18616292	0.8834929
Lh-La	0.8078721	-0.335435	1.95117933	0.3816689
Sa-La	-0.06796	-0.9527929	0.81687281	0.999998
Sb-La	0.8650171	-1.7457602	0.01572604	0.0583297
Se-La	-0.0504	-1.7006218	1.59982177	1
Sa-Lh	0.8758322	-1.876226	0.1245616	0.1353801
Sb-Lh	1.6728892	-2.6696676	-0.6761109	0.0000144
Se-Lh	0.8582721	-2.5732329	0.85668863	0.7925404
Sb-Sa	0.7970571	-1.4821446	-0.1119696	0.0103757
Se-Sa	0.01756	-1.537068	1.57218808	1
Se-Sb	0.8146171	-0.7376869	2.36692107	0.7495506

Appendix 2.3: Tukey multiple comparisons of means of δ13C' among *E. whiteheadi, S. boops, L. australis, L. hectoris, D. meadi, D. hudsoni, S. australis and S. elegans*.

Species	Diff	lwr	Upr	p adj
Dm-Dh	0.8751152	-0.2917925	2.04202283	0.3034494
Ew-Dh	2.674322	1.68557947	3.66306451	0
La-Dh	1.4325461	0.31690516	2.54818704	0.0027325
Lh-Dh	1.1899868	0.01309375	2.36687989	0.0453393
Sa-Dh	2.0907631	1.06744227	3.11408386	0
Sb-Dh	1.4595712	0.43852699	2.48061543	0.000458
Se-Dh	2.2748675	0.75900999	3.79072507	0.0001788
Ew-Dm	1.7992068	1.05618006	2.54223361	0
La-Dm	0.557431	-0.347628	1.46248992	0.5663982
Lh-Dm	0.3148717	-0.6646968	1.2944401	0.976921
Sa-Dm	1.2156479	0.42719216	2.00410367	0.0001019
Sb-Dm	0.5844561	-0.2010427	1.36995483	0.3135288
Se-Dm	1.3997524	0.03143184	2.76807292	0.0407911
La-Ew	1.2417759	-1.9013742	-0.5821776	0.0000006
Lh-Ew	1.4843352	-2.2429474	-0.7257229	0.0000002
Sa-Ew	0.5835589	-1.071002	-0.0961159	0.0072451
Sb-Ew	1.2147508	-1.6973962	-0.7321054	0
Se-Ew	0.3994545	-1.6193886	0.8204797	0.9743828
Lh-La	0.2425593	-1.1604566	0.67533804	0.9927095
Sa-La	0.658217	-0.0521658	1.36859972	0.0920157
Sb-La	0.0270251	-0.6800743	0.73412446	1
Se-La	0.8423214	-0.4825492	2.16719209	0.5246747
Sa-Lh	0.9007763	0.09761609	1.70393641	0.01595
Sb-Lh	0.2695844	-0.5306731	1.0698419	0.9699825
Se-Lh	1.0848807	-0.2919653	2.4617267	0.2429627
Sb-Sa	0.6311919	-1.1812103	-0.0811735	0.0159500
Se-Sa	0.1841045	-1.0640193	1.43222827	0.9699825
Se-Sb	0.8152963	-0.4309616	2.06155427	0.2429627
Sb-Sa	0.6311919	-1.1812103	-0.0811735	0.0122347
Se-Sa	0.1841045	-1.0640193	1.43222827	0.99983
Se-Sb	0.8152963	-0.4309616	2.06155427	0.486313

Appendix 2.3



Tukey HSD test for δ13C' among species *E. whiteheadi (*Ew), (*S. boops* (Sb), *L. australis* (La), *L. hectoris* (Lh), *D. meadi* (Dm) *and D. hudsoni* (Dh), *S. australis* (Sa)and *S. elegans* (Se). Pairwise comparisons with confident intervals of their means differences touching the zero line, indicates that their means differences is not significantly different from zero.

Appendix 3

The following section shows the table for mode, mean 95% credible interval (CI) of feasible contribution of eight prey items into the isotopic mixture of*M. paradoxus, M. capensis, T. capensis* and *L. vomerinus*

Appendix 3.1: mode, mean 95% credible interval (CI) of feasible contribution of eight prey items into the isotopic mixture of *M. paradoxus*

Species	*M. paradoxus*			
	Mode	Mean	Low 95% hdr	High 95% hdr
E.whiteheadi	0.13	0.12	0.04	0.21
D.hudsoni	0.20	0.19	0.11	0.28
D.meadi	0.10	0.10	0.01	0.19
L.australis	0.10	0.10	0.01	0.19
L.hectoris	0.10	0.11	0.00	0.20
S.australis	0.15	0.15	0.04	0.26
S.boops	0.15	0.15	0.05	0.25
S.elegans	0.06	0.07	0.00	0.14

Appendix 3.2: mode, mean 95% credible interval (CI) of feasible contribution of eight prey items into the isotopic mixture of *M. capensis*

Species	*M. capensis*			
	mode	mean	Low 95% CI	High 95% CI
E.whiteheadi	0.18	0.18	0.04	0.31
D.hudsoni	0.05	0.09	0.00	0.19
D.meadi	0.04	0.10	0.00	0.22
L.australis	0.11	0.12	0.00	0.24
L.hectoris	0.02	0.09	0.00	0.21
S.australis	0.16	0.14	0.00	0.27
S.boops	0.16	0.14	0.00	0.27
S.elegans	0.16	0.14	0.00	0.25

Appendix 3.3: mode, mean 95% credible interval (CI) of feasible contribution of eight prey items into the isotopic mixture of *T. capensis*

Species	*T. capensis*			
	mode	mean	Low 95% hdr	High 95% hdr
E.whiteheadi	0.24	0.23	0.08	0.38
D.hudsoni	0.06	0.08	0.00	0.18
D.meadi	0.02	0.08	0.00	0.20
L.australis	0.02	0.10	0.00	0.23
L.hectoris	0.01	0.06	0.00	0.16
S.australis	0.10	0.14	0.00	0.27
S.boops	0.19	0.18	0.01	0.34
S.elegans	0.15	0.13	0.00	0.25

Appendix 3.4: mode, mean 95% credible interval (CI) of feasible contribution of eight prey items into the isotopic mixture of *L. vomerinus*

Species	*L. vomerinus*			
	mode	mean	Low 95% hdr	High 95% hdr
E.whiteheadi	0.22	0.24	0.03	0.43
D.hudsoni	0.01	0.04	0.00	0.12
D.meadi	0.02	0.07	0.00	0.19
L.australis	0.03	0.10	0.00	0.25
L.hectoris	0.02	0.10	0.00	0.23
S.australis	0.18	0.16	0.00	0.33
S.boops	0.02	0.09	0.00	0.22
S.elegans	0.20	0.20	0.00	0.40